ERDC/CERL TR-05-5

US Army Corps of Engineers®
Engineer Research and Development Center

Inhalation Toxicity of Cogenerated Graphite Flake and Fog Oil Smoke in the Brown-headed Cowbird and the Red-winged Blackbird, Size-specific Inhalation Surrogates for the Red-cockaded Woodpecker

Crystal Driver, Robert Fulton, Jennifer Ollero, Mark Clark, Gary Dennis, Brett Tiller, and Harold E. Balbach

January 2005

Construction Engineering Research Laboratory

ERDC/CERL TR-05-5
January 2005

Inhalation Toxicity of Cogenerated Graphite Flake and Fog Oil Smoke in the Brown-headed Cowbird and the Red-winged Blackbird, Size-specific Inhalation Surrogates for the Red-cockaded Woodpecker

Crystal Driver, Robert Fulton, Jennifer Ollero, Mark Clark, Gary Dennis, and Brett Tiller

Pacific Northwest National Laboratory
PO Box 999
902 Battelle Boulevard
Richland, WA 99352

Harold E. Balbach

Construction Engineering Research Laboratory
PO Box 9005
Champaign, IL 61826-9005

Final Report

Prepared for Strategic Environmental Research and and Development Program
Arlington, VA 22203

Headquarters, U.S. Army Forces Command
Fort McPherson, GA 30330

Under CS-507: Threatened, Endangered, and Sensitive Resources

ABSTRACT: The red-cockaded woodpecker (*Picoides borealis*) is an endangered species found on many installations where troop readiness training is conducted. Conducting maneuvers under obscurant cover is important for realistic training. Generators that combine fog oil for visual obscuration with graphite flakes for infrared obscuration are being deployed for training scenarios. The effect of this combination on avian species was unknown. Our data indicate that toxicity of inhaled and/or preened graphite flake and cogenerated graphite flake and fog oil is low and similar to controls for adult cowbird and blackbird surrogates for the red-cockaded woodpecker. No mortality, body weight loss, clinical signs of toxicity, or respiratory function deficits were observed in the graphite flake-only, or cogenerated graphite flake/fog oil-treated birds when compared to controls. Hematological response was normal and no toxic effects in erythrocytes or leukocytes were found. White blood cell counts, spleen weights, and incidence of parasitism and disease were indicative of normal immune function in all treatments. Because repeated exposure that may result in particle overload in the lung has the greatest potential for causing harm to birds, it is suggested that exposures to high concentrations of the aerosol-graphite mixture be limited to fewer than eight within any 2-month period.

Pacific Northwest National Laboratory
Operated by Battelle for the U.S. Department of Energy

Prepared for the U.S. Department of Energy under a related services contract for the U.S. Army Forces Command.

Contents

List of Figures and Tables

Figures

Tables

Conversion Factors

Non-SI* units of measurement used in this report can be converted to SI units as follows:

Multiply	By	To Obtain
acres	4,046.873	square meters
cubic feet	0.02831685	cubic meters
cubic inches	0.00001638706	cubic meters
degrees (angle)	0.01745329	radians
degrees Fahrenheit	(5/9) x (°F – 32)	degrees Celsius
degrees Fahrenheit	(5/9) x (°F – 32) + 273.15.	kelvins
feet	0.3048	meters
gallons (U.S. liquid)	0.003785412	cubic meters
horsepower (550 ft-lb force per second)	745.6999	watts
inches	0.0254	meters
kips per square foot	47.88026	kilopascals
kips per square inch	6.894757	megapascals
miles (U.S. statute)	1.609347	kilometers
pounds (force)	4.448222	newtons
pounds (force) per square inch	0.006894757	megapascals
pounds (mass)	0.4535924	kilograms
square feet	0.09290304	square meters
square miles	2,589,998	square meters
tons (force)	8,896.443	newtons
tons (2,000 pounds, mass)	907.1847	kilograms
yards	0.9144	meters

*_Système International d'Unités_ ("International System of Measurement"), commonly known as the "metric system."

Preface

This study was supported by two projects. The first project funded a series of studies to evaluate the health effects of fog oil aerosols in surrogates for the red-cockaded woodpecker. These studies were conducted for the Strategic Environmental Research and Development Program (SERDP) under project number CS-507 "Threatened, Endangered, and Sensitive Resources: Impact of Smokes and Obscurants on TES." The technical monitor was Dr. Femi A. Ayorinde, SERDP Cleanup and Conservation Program Manager, followed by Dr. Robert W. Holst, Compliance and Conservation Program Manager. The second project, funding this specific research on the inhalation toxicity of graphite flake and fog oil smoke, was conducted for the U.S. Army Forces Command. Dr. Bert Bivings, FORSCOM Natural Resource Program Manager, was the sponsor. Preparation of this ERDC report was funded by U.S. Army Threatened and Endangered Species (TES) research funds, Project P62720A896, Work Unit F474HJ, within the TES Mitigation and Management work package.

The work was performed by the Pacific Northwest National Laboratory (PNNL), Richland, Washington, under Military Interdepartmental Purchase Request MIPR7MR9761045 R97660 S09177. Crystal Driver was the PNNL Principal Investigator. Jennifer Ollero, Mark Clark, Robert Fulton, Brett Tiller, and Gary Dennis also are employed by PNNL. The report was prepared by the Ecological Processes Branch (CN-N) of the Installations Division (CN), Construction Engineering Research Laboratory (CERL). Dr. Harold Balbach, CECER-CN-N, was the CERL Principal Investigator. Steve Hodapp is Chief, CEERD-CN-N, and Dr. John T. Bandy is Chief, CEERD-CN. The associated Technical Director was Dr. William D. Severinghaus, CEERD-CV-T. The Director of CERL is Dr. Alan W. Moore.

Special thanks goes to Elizabeth Keane, Engineering Aide, CEERD-CN-N, who coordinated preparation of the final report.

CERL is an element of the U.S. Army Engineer Research and Development Center (ERDC), U.S. Army Corps of Engineers. The Commander and Executive Director of ERDC is COL James R. Rowan, and the Director of ERDC is Dr. James R. Houston.

1 Introduction

Background

The relatively large expanses of habitat conserved within military installations often support populations of threatened and endangered wildlife species. To ensure that concerns about the continued health of these critical populations do not impede the military mission activities, more accurate knowledge about the actual sensitivities of these species is needed. In the absence of accurate knowledge, regulatory requirements may be established that, while extensive enough to assure population maintenance, have the potential to unnecessarily restrict the military missions of the installations. Troop readiness training is an essential military function of several installations where endangered avian species such as the red-cockaded woodpecker (*Picoides borealis*) occur. Important components of readiness training at these installations are the generation of obscurant material and maneuvers under obscurant cover. Obscurants have long been used to mask movements of troops and mechanized equipment. Of the conventional smokes, the white "smoke" generated from vaporization and re-condensation of liquid fog oil (SGF-2) is an effective obscurant in the visible range and has been used for training for many years. However, the modern battlefield has become more complex, with visual detection being augmented by a wide range of methods for detection and targeting. As a result, effective training for tactical readiness requires experience with both visual and infrared obscurants. Recently, generators have been developed for battlefield uses that are capable of co-emitting fog oil for visual obscuration and graphite to defeat infrared detection.

Because sensitive avian populations must be managed without significant reduction in realistic troop training opportunities, deployment of these smoke generators requires an accurate assessment of the toxicity and health effects of the obscurants in species such as the red-cockaded woodpecker, and others. In a previous study (Driver et al. 2002a) the health effects of fog oil smoke were assessed in wild captive blackbirds, a species used as a surrogate for the red-cockaded woodpecker. In that study, birds were exposed to field-typical levels of airborne fog oil (50 and 100 mg/m^3) and to a near-source concentration of 400 mg/m^3 for 4 hours. Test results showed that, for several potential pathways, the whole body exposures (inhalation, preening, and dermal uptake) did not adversely affect the health or well being of the birds. No mortality, clinical signs of toxicity, weight change, abnormal behavior, or

gross or histopathologic lesions related to fog oil exposure were observed in any of the test birds compared to controls. Therefore, as a result of these studies, a "no observable adverse effect" level was estimated to be greater than 400 mg/m^3 for acute (4-hour) inhalation of fog oil smoke in nonreproductive red-winged blackbirds and birds of similar size-specific ventilation. This interpretation included several listed species of concern to the Army, including the red-cockaded woodpecker.

Primarily through a series of studies sponsored by the Strategic Environmental Research and Development Program (SERDP) (Driver et al. 2002a, b and Driver et al. 2004), some information on the toxicological response of birds to airborne fog oil has been developed. However, virtually no data were available on the inhalation toxicity of airborne graphite flake and mixed aerosols of graphite flake and fog oil on wildlife and, in particular, avian species. Inhalation of graphite flake aerosols has been investigated in human surrogate species (laboratory rodents); however, extrapolation of these data to birds is problematic because of several fundamental differences in the structure and physiology of their respiratory systems.

In contrast to mammals, birds lack alveoli (blind terminal dilations of the airways) and therefore, maintain a continuous circulation of air through the lung instead of an alternating inflow and outflow. This continuous exposure coupled with an exceptionally thin blood gas barrier makes the avian respiratory surface more liable to damage from airborne particulate and chemicals (Scheuermann et al. 1997) and results in increased sensitivity to inspired toxins (Dumonceaux and Harrison 1994). Transport and clearance of airborne particles in the lung also differ markedly between mammals and birds (Klika et al. 1996). In addition to the high efficiency of their respiratory system, birds are generally more susceptible to inhaled toxins because of their rapid metabolic rate, small size, and low body fat content. Because of their elevated metabolic rate and the demands of flight, respiratory rates in birds are generally higher. Consequently, birds ventilate a greater volume of air than those of mammals of comparable size and thereby receive much higher doses of airborne contaminants on a body weight basis (Hill 1994, Schafer 1972, Schafer et al. 1983). Similarly, feather deposition and subsequent oral uptake via preening cannot be estimated from mammalian studies. For bird species with critical populations (threatened and endangered species), exposure and toxicity are best estimated from weight-specific avian surrogates.

Objective

The objective of this study was to evaluate the health effects of graphite flake and mixed aerosols of graphite flake and fog oil in surrogate species for the red-cockaded woodpecker. The information from this study will also be used for general and site-

specific risk assessments and for the management of obscurant generation and training activities. A secondary objective was to characterize the physical nature of the mixed aerosols created by mixing graphite flakes with the fog oil.

Scope

For this study, as for the previous studies in this series (Driver et al. 2002a,b and Driver et al. 2004), exposure is designed to emulate the field conditions for actual use of the obscurants. This was not a classic toxicological study in which a lethal endpoint was sought, and no attempt was made to determine a lethal concentration or duration of exposure. The exposure concentrations and durations, therefore, were planned to parallel those experienced in training exercises on military installations.

Approach

As with earlier studies of the potential effects of fog oil on sensitive listed species, particularly the red-cockaded woodpecker, it was not possible to utilize that species directly in the studies. Neither was any closely related woodpecker species available as a test population, which could be maintained under aviary and laboratory conditions. The brown-headed cowbird (*Molothrus ater*) and the red-winged blackbird (*Agelaius phoeniceus*) were used as species surrogates for the red-cockaded woodpecker because of their similarity in physical size to the red-cockaded woodpecker. The similarity in physical size provided a weight-specific minute ventilation and a feather/body surface area comparable to those of the woodpecker (i.e., similar respiratory and preening/dermal exposure).

The exposure period was patterned after the worst-case scenario usage anticipated in field training. In this study wild cowbirds were exposed to airborne concentrations of graphite flake or to mixed aerosols of graphite flake and fog oil smoke at typical field concentrations (35 mg/m^3 graphite flake; 100 mg/m^3 fog oil) or to levels estimated to be present at less than 100 m from the generator (70 mg/m^3; 120 mg/m^3 fog oil). Red-winged blackbirds were exposed to 35 mg/m^3 of graphite flake or to 185 mg/m^3 graphite flake cogenerated with 300 mg/m^3 fog oil for 30 minutes each day for 4 consecutive days. Control birds were exposed under the same test conditions but without fog oil in the air stream. Two additional groups received a single 1-hour exposure to either 60 mg/m^3 of graphite flake or 60 mg/m^3 of graphite flake in combination with 120 mg/m^3 of fog oil smoke. Birds that were not exposed to control or treatment atmospheres served as aviary controls for comparison of post exposure behavior.

Because the polynuclear aromatic hydrocarbon (PAH) content of fossil fuels may contribute significantly to the toxicity of refined oils and because they can be products of pyrolysis, samples of both the stock fog oil and the fog oil aerosols were collected for PAH analysis. The composition of the fog oil was similar to that of fog oil used in a previous study (Driver et al. 2002a). Fog oil deposition on feathers was also determined to estimate oral exposure from preening. Oral exposure to graphite flake via preening and/or clearance of the respiratory system was estimated from the occurrence of graphite flake in feces.

Mode of Technology Transfer

The information included in this report is one portion of the materials prepared by the Engineer Research and Development Center (ERDC) to assist installation natural resource and Threatened and Endangered Species program managers. The primary means of communicating the hatchling and nestling toxicity information will be through publication in the scientific literature, as well as through the availability of this report. The specific data presented are intended to be used in the preparation of biological opinions related to planned Army actions where the red-cockaded woodpecker (or other similar avian species) is present, and for endangered species management plans (ESMPs), integrated natural resource management plans (INRMPs), and in the preparation of ecological risk assessments involving fog oil smoke and avian species.

This report will be made accessible through the World Wide Web (WWW) at URL:
http://www.cecer.army.mil

2 Methods

Whole body exposures of two species of wild birds to graphite flake and fog oil obscurants were conducted under simulated field conditions. Post-exposure response of the birds to the obscurants was monitored for several weeks and terminal tissue pathology and hematological status evaluated.

Test Animals

Red-winged blackbirds (*Agelaius phoeniceus*) were selected as test subjects because they have long been used as a sensitive test species in environmental toxicology (Schafer 1972, Schafer 1994, Schafer et al. 1983) and have served as a surrogate for the red-cockaded woodpecker in a previous study on the inhalation toxicity of fog oil (Driver et al. 2002a). Nonreproductive female blackbirds from eastern Washington were used in this study because their mean body mass (46.9 g to 48.3 g) is similar to that of the female red-cockaded woodpecker (about 45.9 g to 48.5 g; Table 1) and because female animals are generally more sensitive to test substances (Lipnick et al. 1995). The mean body mass of male red-winged blackbirds in the western United States is about 20 to 25 percent greater than that of red-winged blackbird from other areas of the country (Beletsky and Beadle 1996). The brown-headed cowbird (*Molothrus ater*) was selected as one of the test species for this study because of its history of use in environmental toxicity studies (Van Velzen et al. 1972, Heinz and Johnson 1982, Beyer et al. 1996) and its similarity in physical size to the red-cockaded woodpecker.

The sensitivity of cowbirds to environmental pollutants has been shown to be comparable to, if not greater than that of other passerine species including the red-winged blackbird (Van Velzen et al. 1972, Stickel et al. 1979, Heinz and Johnson 1982, Stickel et al. 1983, Stickel et al. 1984, Beyer et al. 1996). Mean body mass of the male cowbirds used in this study (47.5 g to 49 g) is comparable to the average weight of the male red-cockaded woodpecker (48.1g to 49.8g); however, mean female body mass of cowbirds (37.6 g to 38.8 g) is about 19% less than that of the red-cockaded woodpecker (Table 1). Because of its smaller size, the female cowbird will likely receive a greater dose of obscurants (ventilation of a greater volume of the contaminated air) than the woodpecker on a body weight basis and thereby serve as a conservative surrogate of exposure. Body length of the cowbird averaged about 20 cm (8.0 in.). The average body length of the red-cockaded woodpecker has been re-

ported to be about 22 cm (8.5 in.). Body length is considered a good indicator of size because it, unlike body mass, does not change seasonally but is highly correlated with body mass (Connell et al. 1960, Searcy 1979). The similarity in body size should result in similar weight-specific minute volumes and consequently similarity in inhaled dose (Phalen 1984). Because feather mass and body surface area can be expressed as a function of body mass in birds (Calder and King 1974; Turcek 1966), contamination of these surfaces/exposure routes should also be similar between the two species.

Table 1. Typical body mass for red-cockaded woodpecker and the surrogates used in this study.

Species	Sex	Mass (g)	Region
Red-winged Blackbird [1]	Male	61.0 – 70.5	Ohio
	Female	41.6 – 43.8	
Red-winged Blackbird [2]	Male	63.6 ± 4.43	Pennsylvania [as reported in [3]]
	Female	41.5 ± 2.74	
Red-winged Blackbird	Female	44.3 – 45.6	Benton County, Washington (as used in this study)
Brown-headed Cowbird [4]	Male	47.1 – 48.9	Ontario, Canada
Brown-headed Cowbird	Male Female Combined	48.9 ± 5.7 38.6 ± 4.0 OR 33.9-53.9	Benton County, Washington (as used in this study)
Brown-headed Cowbird [2]	Male	49.0 ± 1.77	Pennsylvania [as reported in [3]] Range for this is 30.5-58.0 (combined)
	Female	38.8 ± 1.93	
Brown-headed Cowbird [5]	Male	47.5 ± 3.7	California
	Female	37.6 ± 3.6	
Red-cockaded Woodpecker [6]	Male	49.8 ± 1.78	Noxubee, Mississippi
	Female	48.2 ± 2.19	
Red-cockaded Woodpecker [6]	Male	48.5 ± 2.46	Fort Polk, Louisiana
	Female	46.9 ± 2.46	
Red-cockaded Woodpecker [6]	Male	48.1 ± 1.81	Savannah River Site Aiken, South Carolina
	Female	45.9 ± 3.18	

(1) Holcomb, L. C. and G. Twiest. 1968. Red-winged Blackbird nestling growth compared to adult size and differential development of structures. Ohio J. Sci. 68: 277–284.
(2) Clench, M. H. and R. C. Leberman. 1978. Weights of 151 species of Pennsylvania birds analyzed by month, age, and sex. Bulletin Carnegie Museum of Natural History no. 5, 87 pp.
(3) Dunning, J. B., Jr. 1993. CRC Handbook of Avian Body Masses. CRC Press, Boca Raton, Florida.
(4) Weatherhead, P. J. and K. L. Teather. 1987. The paradox of age-related dominance in Brown-headed Cowbirds (*Molothrus ater*). Can. J. Zool. 65: 2354–2389.
(5) Fleischer, R. C. and S. I. Rothstein. 1988. Known secondary contact and rapid gene flow among subspecies and dialects in the Brown-headed Cowbird. Evolution 42: 1146–1158.
(6) Jackson, J. A. 1994. Red-cockaded Woodpecker (*Picoides borealis*). In The Birds of North America, No. 85 (A. Poole and F. Gill, Eds.). Philadelphia: The Academy of Natural Sciences; Washington, D.C.: The American Ornithologists' Union.

Source and Husbandry of Test Animals

Wild adult cowbirds were baited to traps in Benton and Franklin Counties, Washington, and transferred to an outdoor aviary at Pacific Northwest National Laboratory (PNNL) 8 weeks prior to testing. Blackbirds were trapped 4 weeks prior to exposure. The birds were housed (at different time periods without overlap of species) in a 9.1-m wide by 15.2-m long by 3.7-m high (30 ft by 50 ft by 12 ft) aviary that was divided into five 3-m wide flight pens. The different species occupied different pens and/or were held at different times. A metal roof covered one-third of each section. Continuous flowing water was provided for drinking and bathing/swimming in each flight pen by capping the end of a 3-m (10-ft) long PVC gutter and providing a constant flow of potable water into one end of the gutter. Spillover ran across the width of each pen to a drain and provided wading area. Wooden rods suspended from the roof frame provided perches for the birds in covered portions of the aviary. Natural and artificial evening roosts consisted of fir (*Abies* spp) and spruce (*Picea* spp) trees placed in planters and arranged around 4-ft high roost boxes. Willow trees (*Salix* spp), spruce trees, arborvitae (*Thuja occidentalis*) and dwarf conifers provided natural cover in the open areas of the flight pens.

Birds were fed a pelleted, complete diet for insect-eating soft-billed birds (Zeigler Bros, Inc., Gardners, PA, Product No. 73534800). Minimum values for crude protein and crude fat content of the semi moist feed were 30.0 percent and 12.0 percent, respectively. Maximum crude fiber was 4.0 percent, maximum moisture was 15.0 percent and the maximum ash content was 8.0 percent. Although the diet was complete, other, varied food items, including meal worms (*Tenebrio molitor*, *Zophobas morio*), larvae of the greater bee moth (*Galleria mellonella*), crickets (*Acheta domestica*), fresh corn (on the cob), commercial mix of wild bird seed, cracked corn wheat, natural millet sprays, berries, and lettuce were also provided for both dietary variety and environmental enrichment.

The aviary perches, roost boxes, water troughs, and floor were disinfected daily. Natural perches were sprayed down with water each day.

The birds were observed by a laboratory animal veterinarian during the quarantine and acclimation period, were found to be in good health, and were released for study at the end of 8 weeks of acclimation.

Bird Identification and Group Assignment

All birds of one species were housed in a single flight pen. To uniquely identify each bird, a 2-mm by 12-mm barcode transponder (Avid Company, Norco, California) was implanted in the pectoralis muscle of each bird with a needle injector. The trans-

ponders were stored in 70 percent ethanol until implanted. A povidone iodine solution was applied to the skin at the implant site as an antiseptic prior to injection of the transponder. Individual birds were identified when needed using a barcode reader (AVID Company, Norco, California). Treatment group designation was made by colored leg bands (National Band and Tag, Newport, Kentucky).

Cowbirds were randomly assigned to test groups using a random numbers generator (Microsoft® Excel). Body weights were measured at 4 weeks prior to testing. The data were analyzed for homogeneity of variance using Bartlett's test and for differences in mean body weights among test groups using one-way analysis of variance (Zar 1974). The results of these statistical tests showed that all the group population variances were homogeneous and no significant differences existed among the mean body weights of all groups ($P > 0.05$). Therefore, the group assignments were retained for the study.

Data from the cowbird study indicated that the capture order of the birds was possibly influenced by some of the treatments. However, this observation could not be sufficiently documented, in part because the relative ease of capture of the birds prior to treatment had not been recorded, and was treated as unknown. Therefore, the group assignments for the subsequent test using red-winged blackbirds were made by capture order. The first 3 birds captured were randomly assigned to one of the 3 experimental groups. This procedure was repeated for each round of capture until each group contained 12 subjects. The groups were then compared on a body-weight basis and the homogeneity of the group population variances were evaluated statistically as described for the cowbirds. No significant differences among the mean body weights of the groups were found.

Exposures

Birds were transferred to the Aerosol Research Facility at Pacific Northwest National Laboratory (PNNL) and exposed to graphite flake or cogenerated aerosols of graphite flake and fog oil within 30 minutes of arrival at the facility. Control birds were similarly collected, transferred, and held in the exposure chambers but were not exposed to the graphite flake or aerosols. A group of birds remained at the aviary and served as untreated aviary controls. For the cowbirds, 2 concentrations of graphite flake were used in the inhalation tests and included a typical (35 mg/m^3) field concentration and a high, near-source concentration of 70 mg/m^3. The toxicity of graphite flake aerosols was tested alone and in combination with fog oil smoke. The cogenerated fog oil smoke concentrations were 100 mg/m^3 and 120 mg/m^3, respectively. Test concentrations were based on predictions from a modified Gaussian plume dispersion model for cogenerated aerosols of graphite flake and fog oil (Driver

et al. 1993a,b). Birds were exposed for 30 minutes each day for 4 consecutive days. In addition to the 30 minutes at the treatment concentration, the birds were exposed to 15 minutes of aerosols as the test atmospheres reached the target test concentrations.

Two single exposure tests were also performed. One group of cowbirds was exposed for 1 hour to 60 mg/m^3 of graphite flake and a second group was exposed for 1 hour to the cogenerated graphite flake (60 mg/m^3) and fog oil (120 mg/m^3). Control birds were handled, transported, and placed in the chambers in a manner similar to treated birds but without exposure to the obscurants.

Red-winged blackbirds were exposed for 4 consecutive days to 30 minutes of graphite flake (35 mg/m^3) or to 185 mg/m^3 graphite flake cogenerated with a 300 mg/m^3 aerosol of fog oil. Controls were placed in the chambers without aerosol exposure.

Test conditions, aerosol characteristics, and bird behaviors were monitored during the exposure tests. After exposure, the birds were returned to their home flight pens in the outdoor aviary. Observations were made for mortality, body weight change, behavioral deficits, and potential signs of toxicity associated with the aerosol exposure during the post exposure period. Cowbirds and blackbirds were sacrificed and examined for hematological, gross and histological lesions at 7 weeks and 5 weeks post exposure, respectively.

Test Material

The test material was obtained from U.S. Army sources and chemically characterized at PNNL. Particle/droplet characteristics of the aerosols were also determined.

Graphite Flake – Source and Composition

Graphite flake was obtained from the Program Manager, Smokes, at Aberdeen Proving Ground, Maryland. The particle size distribution of the flake was characterized as described in Exposure Characterization (page 16).

Energy dispersive X-ray fluorescence was used to analyze two aliquots (200 mg each) of the graphite flake. A comparison standard of 2500 ppm silica was made from 200 mg of cellulose spiked with 50 µL of a 50 µg/mL silica standard (National Institute of Standards and Technology) that was dried to a constant weight. Unspiked cellulose served as the blank matrix control. Data were compared to the trace element recovery in our tests using standard reference materials (coal and oyster tissue) from the National Institute of Standards and Technology.

Fog Oil – Source and Composition

A variety of fog oils have been used to provide white smoke obscurant; however, SGF-2 is the fog oil that has been in use for year-round obscuration needs for the past 20 years (U.S. Army 1986). In 1986, promulgation of military specification MIL-F-12070C specified procurement and use of fog oil with reduced quantities of some of the potentially harmful components of the material (e.g., polynuclear aromatic hydrocarbons). Fog oil used in this study was Lot number 71808 manufactured by American Lubricating Company, Memphis, TN and supplied to PNNL by the National Training Center, Fort Irwin, CA. Upon receipt of the oil, it was bar coded and tracked through the PNNL Chemical Management System.

Because the toxicity of petroleum oils is often related to the amount of polynuclear aromatic hydrocarbons (PAHs) present, samples of fog oil were collected prior to generation and at entry into the test chambers during generation of fog oil smoke both to characterize the stock oil and to determine if the smoke generation process (high heat vaporization) produced an aerosol of different composition than that of the stock oil. Aerosol samples were collected during preliminary generations (airborne concentrations of 120 mg/m^3) on aluminum foil deposition coupons (929 cm^2). Samples were collected for 30 minutes. Unused foil coupons and foil coupons placed in control test chambers receiving output from the generation system described in Aerosol Generation, Fog Oil (page 13) but without fog oil were used as blank controls. All samples were placed in bottles with Teflon-lined lids and stored at -20°C until analyzed for PAH content. Glassware and foil were ashed at 400°C for 24 hours and Teflon-lined lids were rinsed with GC (gas chromatography)-grade hexane and methylene chloride prior to use.

Fog oil samples were extracted with methylene chloride according the National Oceanic and Atmospheric Administration's Status and Trends Program Technical Memorandum NMFS F/NWC 153 (Krahn et al. 1988). Samples were then cleaned using Silica/Alumina (5 percent deactivated) chromatography followed by High Pressure Liquid Chromatography (HPLC) cleanup. Selected dueterated surrogate PAH compounds were added at the beginning of each extraction to assess the efficiency of the method and all results corrected for the recoveries of the surrogates. Extracts were quantified using gas chromatography/mass spectrometry (GC/MS) in the selected ion mode (SIM) following a procedure based on EPA method 8270 (U.S. EPA 1986).

Exposure Chambers

Four Plexiglas chambers were constructed within the PNNL Aerosol Research Wind Tunnel (Figure 1). Each chamber was cubical in shape with a volume of 0.23 m^3 (61

cm on all sides). Three inlet portals, one each for introduction of graphite flake, fog oil aerosols, and dilution air were attached to the upper portions of each chamber. Two additional ports were installed in the chambers to obtain physical samples and allow a small flow to be withdrawn and passed to optical dust sensors for real-time monitoring of aerosol concentrations. No ports were fitted to the control chamber. A single exhaust port was used to control chamber vacuum, and directed aerosols to a wet scrubber/HEPA filtration system prior to venting to the outside. A vacuum gauge was fitted to each exposure chamber to aid in ensuring reproducibility of exposure conditions.

Figure 1. Exposure system used for testing the effects of fog oil and graphite flake aerosols on avian health.

Ambient temperature and humidity of the exposure chambers were maintained at the average values recorded between 09:00 and 11:00 hours at the aviary during a 5-day period prior to the day of exposure. The mean temperature and relative humidity during exposure for the cowbirds were 18.0°C (17.50°C to 18.1°C) and 42.3 percent RH (41.7 percent to 44 percent RH), respectively.

To reduce stress and limit startle responses during testing, the walls of the chambers were made opaque during the exposure tests. One wall of the chamber was moveable and was outfitted with removable perches (Figure 2). To move the birds

out of the chamber and into transfer cages with minimum stress, the perches were pulled out of the chamber side of the wall and affixed to exterior side to be used as handles. The wall was then slowly moved toward the opposing wall, which contained a transfer portal. The transfer cage was covered and the birds returned to their home aviary.

Figure 2. Cowbirds in test chamber prior to initiation of aerosol exposure studies.

Aerosol Generation

Graphite flake was generated using a fluidized bed aerosol generator. Fog oil smoke was generated by recondensation of vaporized oil under conditions simulating field generation. The method of cogeneration of the two obscurants is also described.

Graphite Flake

Graphite flake aerosols were generated by directing the output from a TSI, Incorporated Model 3400 Fluidized Bed Aerosol Generator (FBAG) through a Kr^{80} particle charge neutralizer mounted atop the FBAG, and into the exposure chamber (Figure 3). The air flow rate of the fluidized bed and the graphite flake feed rate from the hopper were adjusted to give the desired concentration within the test chamber. Adjustments typically resulted in a graphite flake-laden airflow rate into the test chamber of 15 Lpm. Graphite flake feed rate from the hopper to the fluidized bed was about 0.8 mg/min using the original factory supplied gearing. To increase the

output of graphite flake aerosol from the TSI Fluidized Bed Generator for the red-winged blackbird cogeneration test, the internal gearing was changed from a gear ratio of 12.5:1 to a ratio of 15:1. The gearing change increased the dry powder feed rate to the fluidized bed chamber by about 20 percent. All other factors remained unchanged. The combination of increased aerosol output and reduced dilution air resulted in the increased graphite flake aerosol concentration attained within the test chamber for the mixed aerosol of graphite flake (185 mg/m^3) and fog oil (300 mg/m^3).

Figure 3. The TSI Model 3400 Fluidized Bed Aerosol Generator used to deliver graphite flake aerosol to the exposure chamber.

Fog Oil

Fog oil aerosols were generated by metering steady rates of liquid fog oil onto a heated immersion element maintained at 600 °C (Figures 4 and 5) and contained within a 1-m long, 2.5-cm diameter stainless steel pipe. The liquid fog oil was vaporized on the element and the vapor was subsequently recondensed as it cooled, forming a fog oil aerosol. Low oxygen carrier gas (a mixture of 96 percent nitrogen and 4 percent air) was used to flush the condensing fog oil vapor through a temperature-controlled region at 300 °C and into a 35-gallon buffer volume with a residence

time of 5 minutes. The oxygen content of the carrier gas was about 0.8 percent, a value typical of the oxygen content present in the exhaust of diesel engines. In the buffer volume, fresh air was mixed with the concentrated fog oil aerosol and the mixture drawn through PVC pipes into the test chambers in the wind tunnel at ambient temperature (18 °C). Valves were used to adjust the flow of aerosol into each of three exposure chambers. Separate valves were used to regulate a flow of fresh air into each chamber. To ensure mixing, restrictions were installed at the aerosol inlet to each chamber. The restrictions caused the fog oil aerosol to jet into the upper regions of the chambers and then quickly mixed to a uniform concentration at the breathing zone height of the birds. The feed rate of the oil was adjusted periodically, based on sensor monitored aerosol concentration to maintain the test concentrations (see Exposure Characterization, page 16).

Figure 4. The fog oil generator used to flash vaporize oil which is delivered to a buffer volume (35-gallon drum), then directed as an aerosol to the manifold and test chambers.

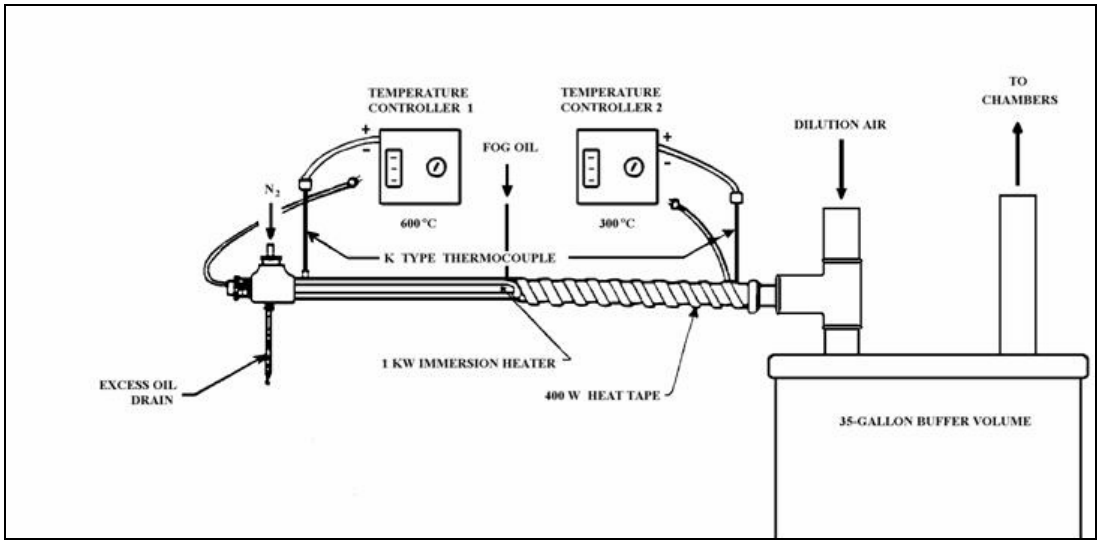


Figure 5. A schematic of the temperature controlled fog oil aerosol generator used in conjunction with the graphite flake generator for mixed aerosols.

Simultaneous Generation of Graphite Flake/Fog Oil Aerosols

Mixed graphite flake/fog oil aerosol generation was performed by initiating the fog oil generator and allowing the chamber concentration to reach equilibrium (about 15 minutes). Fog oil concentrations were monitored in real time using optical dust sensors and confirmed by gravimetric analysis of samples collected on glass fiber filters (see Exposure Characterization, page 16). The exhaust and fog oil input valves were adjusted as needed to achieve the desired concentration. After establishing the fog oil concentration, the powder feed was initiated in the FBAG and the combined aerosols were allowed to reach equilibrium. Samples for mass concentration determination were obtained and the mass contributed by fog oil was subtracted to determine the airborne concentration of graphite flake in the chamber. The airflow rate of the fluidized bed was adjusted as needed to obtain the appropriate concentration of graphite flake.

The entire exposure system, both fog oil smoke and graphite flake, was driven by the exhaust system (energized by the scrubber), with valve lines connected to each chamber. To control flow through the entire aerosol generation and exposure system, careful control of a number of manual valves was necessary to achieve the desired chamber concentrations.

To end a cogeneration, the fog oil pump, the fog oil aerosol intake valve, the carrier gases (nitrogen and air), and the heaters were sequentially shut off. Airflow from the FBAG was turned off and the unit shut down. The fresh air inlet valve was fully opened allowing a few minutes for chamber purge. Following chamber purge, the scrubber was turned off.

Exposure Characterization

In addition to the chemical characterization of the graphite flake and the stock and generated fog oil (see Test Material, page 9), the concentration of graphite flake, the fog oil concentration, and the droplet size distribution of the aerosols to which the birds were exposed were characterized during the exposures. To characterize the preening/dermal exposure of the birds, the deposition rate of fog oil to feathers was determined. Oral exposure to graphite flake from preening and respiratory clearance was estimated from examination of the feces.

Fog Oil Concentration and Droplet Size

In a previous study (Driver et al. 2002a), the vapor component of the fog oil exposures was shown to be minimal. Therefore, particle count and aerosol mass methods were used to determine both the airborne graphite flake and fog oil concentrations during the current exposure tests. The concentration of fog oil aerosol in each of the test chambers was monitored in real time using M.I.E. Model IDS 10 Optical Dust Sensors (Monitoring Instruments for the Environment, Inc., Billerica, Maryland). Actual concentrations were determined from simultaneous gravimetric samples taken by drawing chamber air through preweighed 47-mm high efficiency glass fiber filters (Gelman, Ann Arbor, Michigan) at 1 Lpm for 15 minutes. The filters were weighed to the nearest 0.1 mg on a Mettler Model AE 163 Analytical Balance prior to and after sample collection to determine the mass collected. Airborne fog oil concentrations were reported in mg/m^3. The optical dust sensor values collected during fog oil generation were compared to the gravimetrically derived air concentrations of fog oil. Least squares linear regression was used to test the relationship between the optical dust sensor mV readings and the gravimetric air concentrations (Figure 6). The equation of the linear relationship ($r^2 = 0.98$) was then used to convert the sensor readings into air concentrations values during the exposures.

The particle size distribution of the fog oil aerosols was measured during prestudy testing, independent of graphite flake aerosol. Samples were collected from fog oil aerosols at a concentration of approximately 160 mg/m^3 using an Andersen cascade impactor operated at a flow rate of 20 Lpm (Figure 7).

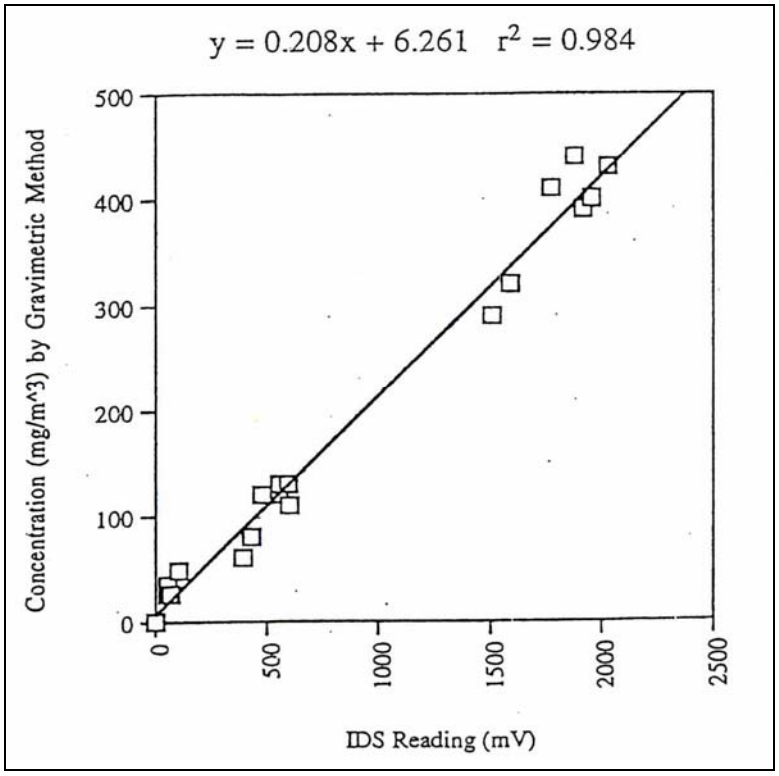


Figure 6. Relationship between millivolt readings of the optical dust sensor and the mass of fog oil collected on glass fiber filters.

Figure 7. Fog oil and graphite flake aerosol collected on cascade impactor filter inserts.

Graphite Flake

The concentration of graphite flake aerosol in the test chamber was also monitored in real time using an optical dust sensor. Since graphite flake is used under field conditions to obscure the light path, the optical dust sensor was considerably more insensitive to graphite flake aerosol concentration compared to fog oil aerosol. Actual concentrations were measured using high efficiency glass fiber filters (GFF). The particle size distribution of the graphite flake aerosol was measured independent of fog oil aerosol during the preexposure testing phase using an Andersen cascade impactor operated at a flow rate of about 20 Lpm.

Cogenerated Graphite Flake and Fog Oil Smoke

Particle size distribution measurements were obtained in a similar manner for the simultaneously generated graphite flake/fog oil aerosols.

Fog Oil Deposition on Feathers

An estimate of the amount of fog oil available for ingestion through feather preening was made by placing taxidermic birds in the control and test chambers during a 1-hour generation. The feathers of the birds were then removed and placed in solvent-rinsed jars with Teflon-lined lids and stored at -20 °C until analyzed for fog oil content. Ions 43 and 57 which are predominant ions in alkanes, the principle component of fog oil, were used to "finger print" the fog oil on the feathers by GC/MS as described in Test Material (page 9). Ion 57 was used as the quantifying ion. However, some late eluting compounds (probably natural feather oils) that had ions 43 and 57 were found in the feather extracts. Potential exaggeration of the fog oil value from this matrix was circumvented by summing only the area between 20 and 40 minutes, which appeared to account for all the fog oil in the feathers while excluding the natural oils.

Fog oil was reported on a per-gram-of-feathers basis and the amount deposited on the bird estimated from the equation:

$$Y = 0.09M^{0.95} \qquad \text{[Equation 1]}$$

where:
Y = the plumage weight in grams, and
M = the average body weight in grams (Turcek 1966, Walsberg and King 1978).

Estimate of Graphite Flake Ingested by Cowbirds

Graphite flake ingestion from two sources, preening and swallowing flake cleared from the respiratory system via the mucociliary escalator, was estimated from the amount excreted in the feces of the cowbirds. Feces from each of the groups were composited daily during the post exposure holding and transport period. Three aliquots of the composited fecal material from each group were weighed and suspended in distilled water and 0.5 ml of each of the suspensions transferred to a Petroff Hausser Counting Chamber. Graphite flakes were viewed under a brightfield microscope and the number of flakes counted. Samples were compared to clean (no fecal matter) dilutions of graphite flake to ensure identification of the flakes from background material. A rough estimate of the mass of flakes ingested by each bird was calculated from the density, average dimensions, and the number of flakes present. The value was then divided by the number of birds in each group. Flake ingestion was estimated to be roughly equivalent to the fecal output and expressed on a per-bird basis. The calculation was made assuming fecal and flake output was constant and that the graphite flake was chemically resistant to dissolution within the gastrointestinal tract.

Estimate of Graphite Flake Deposition in Lung

Worst case deposition of graphite flake was estimated from the equation:

$$D = CVT(10^3 \text{ ml/L})(m^3/10^6 \text{ mL})\ (1/M) \qquad \text{[Equation 2]}$$

where:

D = the amount of graphite flake deposited in the lung (expressed in mg/g lung),
C = the airborne concentration of the flake in mg/m^3,
V = the minute volume of air breathed,
T = the cumulative duration of exposure in minutes, and
M = the mean lung mass for the species.

The respiratory minute volume (V) for resting birds was estimated from the equation:

$$V = 0.291M^{0.94} \qquad \text{[Equation 3]}$$

where:

M = the body mass of the bird in kg (Bernstein 1987).

Response Measures

The response of the birds to the graphite flake and mixed graphite flake and fog oil aerosols was monitored by measures of mortality rate, clinical signs of toxicity and stress, body weight changes, behavioral changes, hematology and blood chemistries, and the gross and histopathologic examination of organs and tissues.

Mortality and Clinical Signs of Toxicity and/or Stress

Observations for clinical signs of toxicity, mortality, and moribund birds were recorded twice daily (before 10:00 hours and after 15:00 hours) during normal workdays and on weekends. In addition to overall condition, observers were required to check for, but were not limited to, the following clinical signs: hyperactivity/hypoactivity, emaciation, abnormal posture, alopecia, swelling, hypothermia, dehydration, tremor, excessive preening, cloacal stain, feather loss, fecal discoloration, eye irritation or sunken appearance, fluffed feathers, excessive face and beak rubbing, and ataxia.

Particular consideration was given to clinical signs of upper and lower respiratory disease. Clinical signs of upper respiratory disease for which observers looked included open-mouth breathing, gular flutter, nasal discharge, change in voice, exercise intolerance, dypsnea, head shaking, stretching the neck, yawning, and epiphora. Clinical signs of lower respiratory lesions for which the observers looked were tail bobbing, loss of voice, change in vocalization, labored respiration, and coughing.

Gross Lesions and Histopathology

All birds were sacrificed at 7 weeks post exposure. A gross necropsy was performed on each bird. Birds were sacrificed by carbon dioxide asphyxiation. Immediately following sacrifice, the intestines were excised, examined, and injected with l0 percent buffered formalin to prevent autolysis. All major organs were examined for gross lesions. The breast muscle was examined for atrophy and the terminal body weight, general body condition, and presence of body fat recorded. Air sacs were viewed for lesions and clarity of tissue. The skin, lung, trachea, heart, liver, spleen, proventriculus, pancreas, small and large intestine, gizzard, kidney, and gonad were excised and preserved in 10 percent buffered formalin. The trachea and lung were perfused with formalin prior to storage in sample jars containing buffered formalin. The sex of the bird and its reproductive status were confirmed during necropsy. Tissues were prepared for histologic examination and submitted for reading by a veterinary pathologist. Tissues were labeled so that specific treatment of individual birds was not identifiable by the pathologist.

Clinical Pathology - Hematology and Blood Chemistry

As measures of stress and the impact of the obscurants on immune response, leukocyte numbers and morphology in peripheral blood were examined. Because hemolytic, regenerative anemia is well documented in birds exposed to petroleum products (Leighton 1982), erythrocyte morphology and packed cell volume (PCV) were measured to determine the incidence of anemia among treated birds and the ability of the bone marrow to respond to the anemic state.

Cytologic examination of the peripheral blood of both cowbirds and blackbirds was performed at necropsy. Blood smears were obtained immediately following blood collection to preserve cell morphology and were made using a standard 2-slide wedge technique. Differential white cell counts were performed on blood smears stained with a Wright Giemsa stain. Total white cell counts were performed using Natt Herrick's stain with a hemocytometer. Cells were stained and classified according to Dein (1984). Leukocyte, thrombocyte, and erythrocyte morphology were evaluated for signs of toxicity (degeneration) and the degree of toxicity reported subjectively on a scale of +1 to +4. A value of +1 reflects a slight damage and +4 indicates severe damage. Abnormal leukocyte morphology was described by increased cytoplasmic basophilia, vacuolation, immature or abnormal cytoplasmic granules, degranulation, and nuclear karyolyis. Toxic changes in thrombocytes were noted by diffuse eosinophilic cytoplasm, spindle shaped cells and immature cells. Erythrocyte toxicity was characterized by presence of immature erythrocytes, polychromasia, variations in nucleus location and shape, cytoplasmic basophilic stippling, and agglutination.

Hematocrit was determined on whole blood samples from the red-winged blackbirds only. The packed cell volume was obtained by centrifugation of a blood filled microhematocrit tube at 12,000 G for 5 minutes.

Several clinical chemistries were also conducted on the peripheral blood. Endpoint metabolites including calcium, cholesterol, glucose, total protein, albumin, globulin, and uric acid were measured to assess the functional integrity/capacity of the major organs. Blood was collected from each bird at necropsy by heart stick and the sample transferred to a MICROTAINER® brand serum separator tube and centrifuged at 14,000 rpm for 10 minutes. The clot was removed and the serum stored at 80 °C until analysis. Metabolite concentrations were determined using a Hitachi® model 7170 clinical analyzer and the reagents and procedures standardized for use with the analyzer. Cholesterol, glucose, and uric acid were analyzed enzymatically using microbial cholesterol esterase, hexokinase, and uricase, respectively. Dye binding techniques were used for the calcium and albumin determinations and total protein was determined by biuret method.

Cell counts and metabolite concentrations of treated birds were compared to control values and to published reference intervals that define the normal limits for healthy populations of passerine species (Altman et al. 1997). Among groups, determination of significant differences concerning the same parameter was measured using one-way analysis of variance (ANOVA) at an alpha level of 0.05 and the means separated by Dunnett's t-test. When data were nonparametric as determined by Barlett's test of equal variance ($p \leq 0.05$), the Kruskal Wallis test was used (Zar 1974). Those blood parameters expressed in a ratio were analyzed using an arcsine transformation. The software system SAS® (SAS Institute, 1995, Cary, North Carolina) was used to conduct all statistical analyses.

Body Weight and Organ Mass

Body weights were measured to the nearest gram on a Sartorius QS4000 balance at 4 weeks prior to exposure, at exposure (Day 0), and at days 14, 24, 36, and 46 post treatment. Organ weights were obtained at necropsy. The tissue mass was determined to the nearest milligram using a Mettler AE260 Analytical Balance. Prior to each weighing session, the scale and balance calibrations were checked using a calibrated Troemner (Philadelphia, Pennsylvania) metric weight set (2 mg to 100 g). The balance and weight set calibrations are traceable to National Institute of Standards and Technology standards. Body weight data were analyzed using one-way analysis of variance (ANOVA) to test for differences in mean body weights among test groups. Organ weights were expressed on a percentage of body weight basis and an arcsine transformation applied to this proportion. The arcsine transformed data were also tested by ANOVA. Treatment group means were compared to the mean weight of the control group by Dunnett's t test (Zar 1974). Statistical analyses were made using the software system SAS® (SAS Institute, 1995, Cary, North Carolina).

Behavior

Behavioral observations were made twice daily (see Mortality and Clinical Signs of Toxicity and/or Stress, page 20) for 1 week prior to fog oil exposure and for Days 0 through 5 and Days 13, 23, 35, and 45 post-exposure. Observers were outfitted with Swarnovski/Habicht SL 10 X 50 binoculars. Birds were observed 30 minutes in the morning and 30 minutes in the late afternoon and for 30 minutes after return to the aviary following exposure. In addition to general signs of stress or disease (see Mortality and Clinical Signs of Toxicity, page 20), the observers were instructed to look for behaviors related to feather cleaning and those behaviors that have been observed in birds dosed with crude or refined oils (Hartung 1967; Croxall 1977; Fleming et al. 1982). These behaviors included thermal deficient behaviors such as increased feeding, hiding, and lethargy, and postural abnormalities wherein the head

is held close to the body and the breast held lower than in normal stances during walking or standing. The number of birds engaged in each of these behaviors during the observational periods was compared to the number of control birds displaying similar behaviors during the same period. A video record was made of any prominent behavioral changes as they occurred.

After the first 2 recaptures of the cowbirds for body weight measurement, it appeared that birds from some treatment groups were more easily caught than birds from control groups. Therefore the order of capture was recorded for each of the subsequent capture events. Although the group identification was always recorded, the individual bird identity record related to capture order was not complete for two of the recapture events. At the end of the study, the birds were categorized by ease of capture for each capture event and placed in 1 of 5 capture categories for any given day of capture. The categories represented the first 20 percent captured, the second 20 percent captured, and so forth.

Because conclusions from these data were greatly impacted by omissions in the data collection (no pre-exposure capture order, no data during the exposures period and immediately post exposure, and missing data tracking individual bird capture), the subsequent design of the red-winged blackbird exposure study included pre-exposure capture events and individual as well as group identification for all post-exposure recaptures.

In addition, the group assignment method was altered so those members of capture categories were distributed equally among groups. The capture data were tabulated in contingency tables and analyzed by the Chi-square statistic (Zar 1974). The frequency of capture for each treatment group was compared to the control group and the alpha value reported.

Weather Measurements

Wind speed, temperature, relative humidity, and rainfall were monitored throughout the acclimation and test phases of the studies using a Met One (Grants Pass, Oregon) weather station equipped with a Model 034 wind sensor.

3 Results and Discussion

Graphite flake was found in the lung and feces of exposed birds. More flakes appeared to be deposited in the lungs of birds exposed to graphite flake without fog oil. However, greater numbers of flakes were seen in the feces of birds exposed to graphite flake cogenerated with fog oil. An immune response to the flake was only observed in birds exposed to graphite flake without fog oil. These data suggest that flake deposition in the lung was reduced when flake was cogenerated with fog oil and may be related to coagulation of the flakes in the presence of fog oil.

Generation of fog oil results in changes in chemical composition of the oil. Primarily, the concentration of acutely toxic naphthalene was reduced by about 98 percent and the number and concentration of high molecular weight PAHs was increased. However, the airborne concentrations of the high molecular weight PAHs were very low to nondetectable. Estimated oral doses of oil and PAHs from preening were much lower than levels reported to cause hypothermia, gross pathologies, tissue damage, or death.

Exposure Characterization

Mean airborne concentrations of graphite flake and fog oil and the mean droplet size of the exposure aerosols were comparable to those measured in or predicted for field tests (Driver et al. 1993a,b). The high exposure concentrations were similar to near source values. Fog oil and PAH exposure appeared to be low for both inhaled and oral (preened) routes. Graphite flake ingestion from preening or from swallowing mucous expelled from the respiratory tract was less than 1 mg/kg body weight. Cogeneration with fog oil appeared to reduce the particle loading of the lung.

Graphite Flake and Fog Oil Concentration and Droplet Size

The airborne concentration of test material for each cowbird exposure is shown in Table 2. For cowbirds, the mean graphite flake concentrations over the 4 consecutive days of exposure were 34 mg/m^3 and 69 mg/m^3 for the low and high graphite only exposures, respectively. The mean graphite flake concentrations during the cogeneration tests were also 34 mg/m^3 and 69 mg/m^3. Fog oil concentrations for these tests were 121 mg/m^3 and 103 mg/m^3, respectively. In the single 1-hour exposures, the graphite concentrations were 59 mg/m^3 for the graphite flake only expo-

sure and 55 mg/m^3 when generated simultaneously with fog oil. The fog oil concentration during the 1-hour single exposure was 131 mg/m^3.

Table 2. Concentration of test material during the cowbird exposures.

Test Aerosol	Exposure Day	Measured Total Concentration (mg/m^3)	Std Dev (mg/m^3)	Coeff Variation (%)	Estimated Component Concentration Within Mixed Aerosols	
					Fog Oil (mg/m^3)	Graphite Flake (mg/m^3)
GF only [(a)]	0	55.1	8.6	15.6	---	---
GF only	0	26.6	3.6	13.4	---	---
GF only	1	72.2	10.9	15.1	---	---
GF only	1	35.1	2.5	7.1	---	---
GF only	1	72.2	10.9	15.1	---	---
GF only	1	35.1	2.5	7.1	---	---
GF only	2	68.9	6.9	10.0	---	---
GF only	2	32.5	3.6	10.9	---	---
GF only	3	79.72	15.15	19.0	---	---
GF only	3	40.03	6.55	16.4	---	---
GF/FO [(b)]	0	211.9	35.4	16.7	156.8	55.1
GF/FO	0	151.3	3.7	2.5	124.7	26.6
GF/FO	1	182.1	5.5	3.0	109.9	72.2
GF/FO	1	138.5	3.5	2.5	130.4	35.1
GF/FO	2	162.1	0.6	0.4	93.2	68.9
GF/FO	2	148.6	18.0	12.1	116.1	32.5
GF/FO	3	202.1	15.07	7.5	122.4	79.7
GF/FO	3	106.3	6.82	6.4	66.3	40.0

[(a)] Unmixed aerosol of graphite-flake only.
[(b)] Mixed (cogenerated) aerosol of graphite-flake and fog oil.

The test concentrations for the blackbird exposures are shown in Table 3. Blackbirds were exposed to a mean concentration of 37 mg/m^3 of graphite flake during the graphite only exposures. The mean graphite concentration for the mixed aerosol was 185 mg/m^3. The mean fog oil concentration in the mixed aerosol was 298 mg/m^3.

Table 3. Concentration of test material during the blackbird exposures.

Test Aerosol	Exposure Day	Measured Total Concentration (mg/m^3)	Std Dev (mg/m^3)	Coeff Variation (%)	Estimated Concentration of Components in Mixture	
					Fog Oil (mg/m^3)	Graphite Flake (mg/m^3)
GF [(a)]	0	38.03	7.87	20.69	---	---
GF	1	37.20	7.33	19.70	---	---
GF	2	34.72	6.88	19.82	---	---
GF	3	36.38	8.96	24.63	---	---
GF/FO [(b)]	0	633.27	108.42	17.12	357.14	220.90
GF/FO	1	511.74	58.31	11.39	287.70	179.18
GF/FO	2	656.42	172.48	26.28	370.37	228.84
GF/FO	3	314.98	65.21	20.70	175.26	111.78

[(a)] Unmixed aerosol of graphite-flake only.

[(b)] Mixed (cogenerated) aerosol of graphite-flake and fog oil.

The aerodynamic mass median diameter (AMMD) of the fog oil smoke droplets was 1.1 µm with a geometric standard deviation (GSD) of 1.7 (Figure 8). The graphite flake size distribution when generated without fog oil is shown in Figure 9. The flake size was 3.5 AMMD with a GSD of 2.1. A slight bimodality was apparent in the graphite flake distribution. These droplet distributions are typical of oil aerosols generated in the field (Driver et al. 1993a,b).

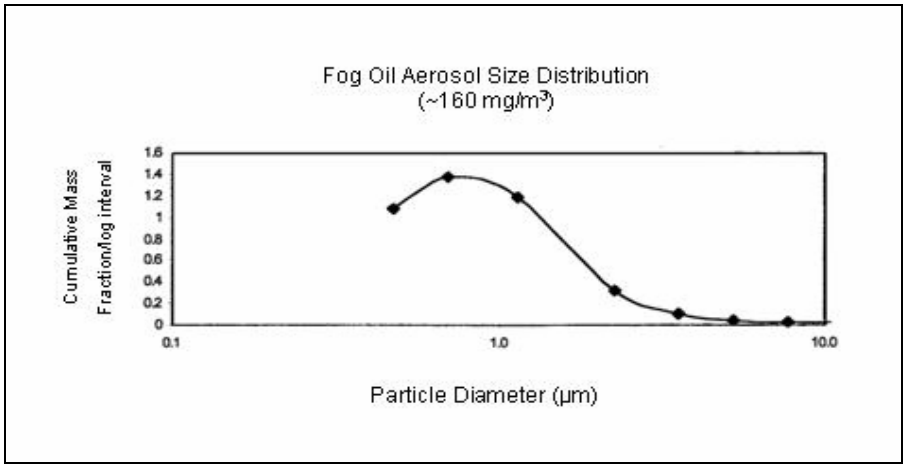


Figure 8. Particle size distribution of fog oil smoke.
The AMMD was 1.1 µm with a GSD of 1.7.

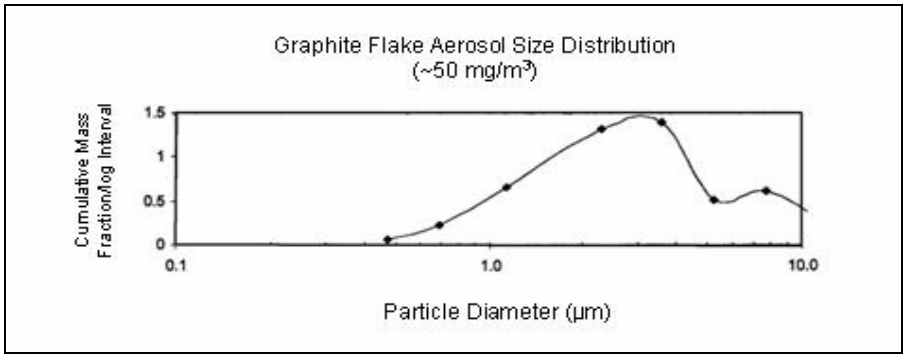


Figure 9. Particle size distribution of graphite flake. The AMMD was 3.5 µm with a GSD of 2.1.

The particle size distribution measurements for the cogenerated graphite flake and fog oil smoke are presented in Figure 10. The size distribution characteristics of each of the two aerosols are evident from the graph. Figure 11A plots the data as if the distribution for the combined aerosols was log normal (i.e., as if it were a single component aerosol). Because the distribution is a summation of two separate log normal distributions, the cumulative distribution curve has a characteristic S shape (Figure 11A). Application of a best-fit plot of the data shows an AMMD of 2.1 µm and a GSD of 2.3 for the combined aerosol data. However, the overall mass median diameter of the combined aerosol may be misleading because it is greatly influenced by the relative concentration of the separate aerosols and does not account for changes in mean particle size of the component aerosols. To determine the effect of cogeneration on particle size, the two distributions were separated from the cumulative distribution by an iterative process described by Moss and Cheng (1995). The resulting best-fit plots for each distribution are shown in Figure 11B. The AMMD of the small particles (fog oil) in the mixed aerosol was about 0.8 µm with a GSD of 2.7. The AMMD of the graphite flake was larger (4.2 µm) when generated with fog oil than when generated alone (3.5 µm). The increased size suggests particle growth as a result of collisions between the fog oil droplets and/or enhanced flake-to-flake agglomeration in the presence of oil coated flakes.

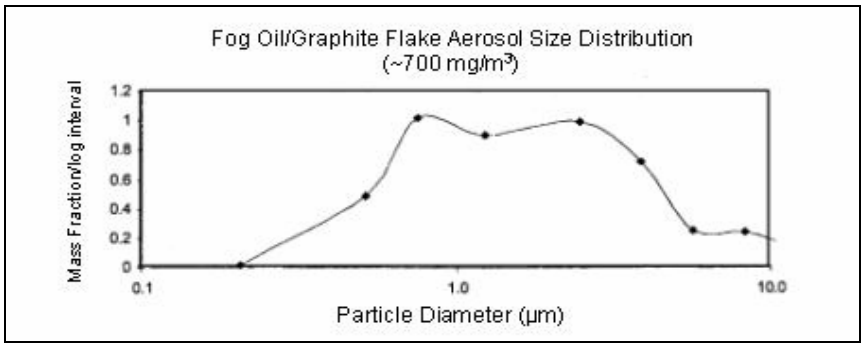
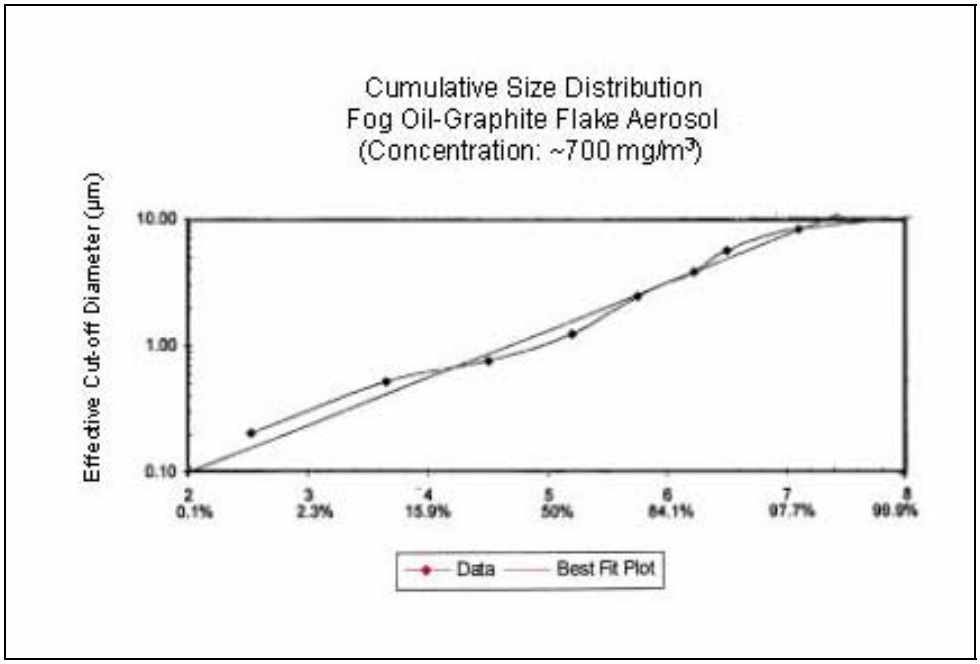
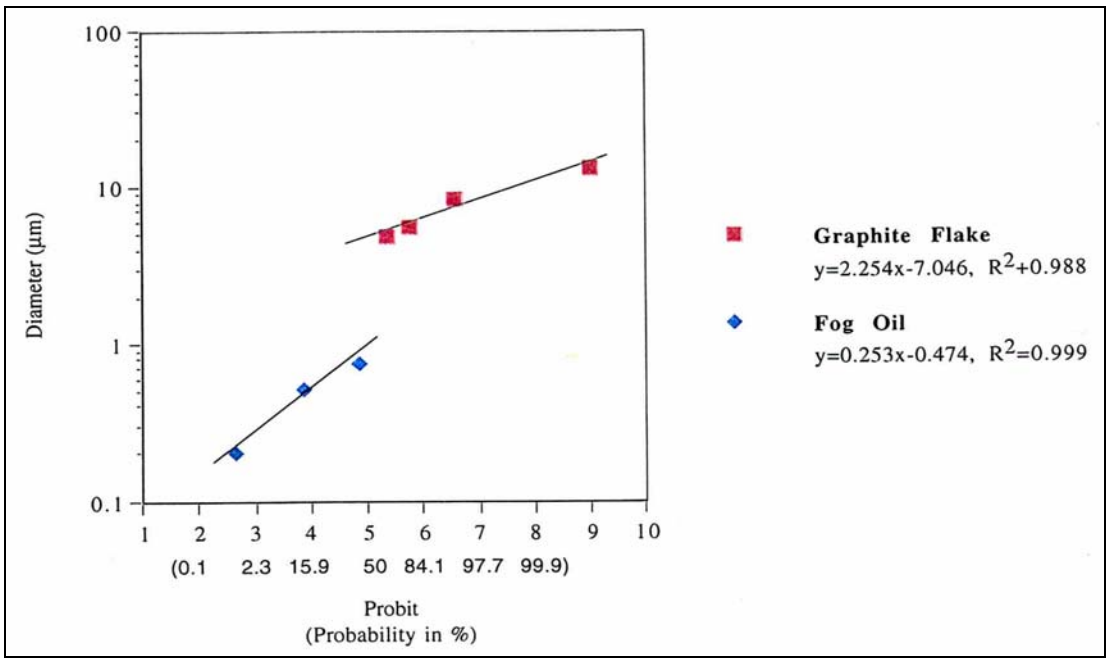


Figure 10. Aerosol size distribution of cogenerated graphite flake and fog oil smoke.



A. Size distribution plotted as a single aerosol, but demonstrating a cumulative summation of two log-normal distributions.



B. Best-fit curves for the separate size distributions.

Figure 11. Size distribution of fog oil droplets and graphite flake in cogenerated aerosol. The horizontal axis displays both probit (whole number) and cumulative mass percentage values.

Elemental Composition of Graphite Flake

Elemental composition of the graphite flake obscurant is shown in Table 4. The graphite flake contained about 0.1 percent silica, an element implicated in fibrogenic disease of the lung. This level of silica, however, is below concentrations that induce silicosis in test animals exposed to carbon dusts (Thomson et al. 1986 and 1987, Anderson et al. 1989). Other elements detected in the flake totaled less than 0.09 percent of the graphite composition. Results are also shown for our analyses of the National Institute of Standards and Technology (NIST) coal standard and the cellulose comparison sample.

Table 4. Elemental composition of the graphite flake obscurant.

Graphite Flake				Reference Standards	
Elements	Rep1	Rep2	Cellulose Spike	Coal	Percent Recovery
Si	950*	1179	2700	36000	113
P	ND (a)	ND (a)	295	ND (a)	326
S	117	97	ND (b)	14230	105
Cl	ND (c)	ND (c)	58	1037	128
K	57.3	69.9	7.5	2920	121
Ca	267	285	46.9	4660	123
Ti	56	61.6	ND (d)	946	95
V	10.2	10.2	ND (e)	45	129
Cr	15.9	21.5	ND (f)	19.1	96
Mn	4.1	3.9	ND (g)	39.9	106
Fe	217	203	5.5	8420	107
Co	ND (h)	ND (h)	ND (h)	18.0	267
Ni	2.9	3.47	1.03	16.1	114
Cu	48	79.4	ND (i)	18.8	104
Zn	ND (j)	ND (j)	ND (j)	33.9	95
Ga	ND (k)	ND (k)	ND (k)	5.25	74
Hg	ND (l)	ND (l)	ND (l)	ND (l)	NA
Se	ND (m)	ND (m)	ND (m)	2.1	70
Pb	ND (n)	ND (n)	ND (n)	10	42
As	ND (o)	ND (o)	ND (o)	4.78	82
Br	ND (p)	ND (p)	ND (p)	17.5	92
Rb	ND (q)	ND (q)	ND (q)	20	100

* Values are in ppm.

(a) P < 450; (b) S < 50; (c) Cl < 41; (d) Ti < 16; (e) V < 10; (f) Cr < 7.2; (g) Mn < 2.5; (h) Co < 18; (i) Cu < 1.1; (j) Zn < 1.1; (k) Ga < 1.3 (l) Hg < 3.9; (m) Se < 0.94; (n) Pb < 2.7; (o) As < 1.2; (p) Br < 0.87; (q) Rb < 1.1.

Concentration of Polynuclear Aromatic Hydrocarbons in Fog Oil Aerosols

The composition of the fog oil before generation is shown in Table 5. Concentrations of lower and higher molecular weight PAHs in the pregeneration oil totaled 266 ppm and 32 ppm, respectively. These concentrations are low and are less than half of the PAH concentrations found in the pregeneration fog oil from an earlier experiment (Driver et al. 2002a). No PAHs were detected in post-generation samples. Generation may not have been long enough to collect sufficient samples for PAH detection (but represents the amount to which the birds were exposed). In the previous study, the composition of the fog oil changed during generation, producing an aerosol with greatly reduced levels of naphthalene (a compound very toxic to birds) and minimal concentrations of higher weight PAHs. Because the airborne concentration of fog oil in the previous study was about 4 times greater than that of the current study and the levels of the toxic components were low; it is unlikely that the cowbirds in the current study were exposed to harmful levels of PAHs.

No fog oil was found (< detection limit of 1 µg/g) on the feathers of taxidermic birds exposed for 30 minutes to airborne fog oil concentrations of 120 mg/m^3 or less. A fog oil deposition rate of 2.8 µg/g feathers/min to 5.5 µg oil/g feathers/min has been determined in a previous study (Driver et al. 2002a) for birds sitting in aerosols of about 400 mg/m^3. From these data a rough estimate of the deposition rate of fog oil in a 100 mg/m aerosol would be about 0.7 µg/g feathers/min to 1.4 µg/g feathers/min.

With deposition rates of fog oil on feathers about 0.7 µg/g feathers/min to 1.4 µg/g feathers/min, oil accumulations of 0.09 mg/g of feathers to 0.19 mg/g of feathers could be expected for birds exposed to 100 mg/m^3 for 30 minutes. Typically, birds contaminated in oil spills preen between 20 and 50 percent of the oil deposited to feathers within an 8-hour period (Hartung 1963, 1967). Assuming a worst-case scenario that birds would ingest, from preening, 100 percent of the feather-deposited fog oil and using Equation 1 (page 18), the accumulated dose over 4 days for red-cockaded woodpeckers exposed for 30 minutes to fog oil concentrations of 100 mg/m^3 is estimated to be 26 mg/Kg to 48 mg/Kg in adult males. These doses are considerably lower than those found to be harmful to birds ingesting unrefined oils (Hartung and Hunt 1966, Chia 1971). Significant pathologies from preening unrefined petroleum oils have been observed only in waterfowl that have obtained doses of at least 1 ml/Kg (Hartung and Hunt 1966, Chia 1971). No toxic or gross lesions were associated with fog oil exposure in the current study. Therefore, preening does not appear to be a toxicologically significant route of uptake for concentrations of aerosolized fog oil used in military exercises.

Table 5. Fog oil composition before and after generation.

Constituent	Conc (µg/g Fog Oil)	
	Before Generation	After Generation[a]
Lower Molecular Weight PAHs:		
Naphthalene	78.5	1.8
1-methyl naphthalene	98.3	<MDL[b]
Biphenyl	22.2	<MDL
2, 6 dimethyl naphthalene	110	<MDL
2,3,5 trimethyl naphthalene	71.9	<MDL
Acenaphthylene	<MDL	2.5
Acenaphthene	8.9	3.2
Fluorene	32.4	21.5
Dibenzothiophene	335	264.0
Phenanthrene	165	162.0
1 methyl phenanthrene	50	<MDL
Anthracene	23.9	15.2
Fluoranthene	14.6	27.2
Higher Molecular Weight PAHs:		
Pyrene	9.1	40.2
Benzo(a)anthracene	<MDL	14.5
Chrysene	52.7	57.2
Benzo(b)fluoranthene	<MDL	<MDL
Benzo(k)fluoranthene	<MDL	<MDL
Benzo(e)pyrene	<MDL	<MDL
Benzo(a)pyrene	<MDL	<MDL
Perylene	<MDL	<MDL
Indeno(123-cd)pyrene	<MDL	<MDL
Dibenzo(a,h)anthrancene	<MDL	<MDL
Benzo(g,h,I)perylene	<MDL	<MDL

PAH Deposition on Feathers and Estimated Oral Dose

It has been suggested that the differences in toxicity observed for various crude oils and other petroleum products in birds are due to the variability in the aromatic composition of the oils (Leighton 1982). Because the toxicity of petroleum oil is largely attributed to its PAH content (Hoffman 1979; Patton and Dieter 1980; Peakall et al. 1981, 1982), the oral dose of the individual and combined PAHs in the fog

oil were estimated. However, no detectable levels of either the concentrations of low molecular weight (2 and < 4 benzene rings) or high molecular weight (>4 benzene rings) PAHs were found on feathers of taxidermed birds exposed to fog oil aerosol or on foil deposition coupons after 30 minutes of exposure. Assuming a linear relationship between the deposition rate (ng of total PAH/g feathers/min) of PAH on feathers and the airborne fog oil concentration as described in Driver et al. (2002a), the oral uptake of low molecular weight PAHs from preening contaminated feathers was estimated. For an airborne concentration of 100 mg/m^3, the rate of PAH deposition would be about 3.5 ng/g feathers/min. Assuming the worst case scenario described in Concentration of Polynuclear Aromatic Hydrocarbons in Fog Oil Aerosols, (page 30) a male red-cockaded woodpecker could receive an oral dose of low molecular weight PAH of about 0.008 mg/Kg after 30 minutes of exposure. This dose is considerably lower than the 25 mg/Kg "no effect" dose estimated for PAHs in wild birds (Driver et al. 2002a).

Estimate of Oral Exposure to Graphite Flake

Birds appeared to be swallowing a large number of graphite flakes (Table 6); however, ingestion of the flake on a mass basis (via preening and from respiratory clearance) was minimal (Table 6). Cumulative oral doses were estimated to be less than 85 µg/Kg for the cowbirds over the first 4 days of the test. Of interest is the pattern of greater excretion of graphite flakes by birds exposed to fog oil during the graphite exposures (Table 6). Birds exposed to the cogenerated aerosols excreted about 20 percent more flakes per gram of feces, and they also excreted about 40 to 100 percent more total flake mass than birds exposed to aerosols of graphite flake alone. These data parallel the diminished deposition of graphite flake in the deep lung of birds exposed to the cogenerated aerosol and the leukocyte response in these birds (see Histopathology, page 38 and Clinical Pathology – Hematology and Blood Chemistry, page 21). The increase in excreted flakes may be due to agglomeration of the flakes with fog oil droplets and flakes in the presence of fog oil. The resulting increase in particle size would likely lead to greater deposition of flakes on feathers and therefore greater ingestion from preening. The increase in AMMD would also result in less penetration to lung and greater deposition in the upper regions of the respiratory tract where the material would be more readily removed via the mucociliary escalator.

Graphite flake within the feces of a bird exposed to a field typical concentration of graphite flake is shown in Figure 12.

Table 6. Number of graphite flakes in feces of cowbirds exposed to graphite flake aerosols or cogenerated aerosols of graphite flake and fog oil for 4 days and estimated mass of ingested flake per bird.

Treatment	Number of Flakes in Feces (Flake/g Feces)	Mass Graphite Flake Ingested (ng/bird)
Control	0	0
Graphite Flake		
35 mg/m^3	3,062,500	1,647
70 mg/m^3	2,864,583	1,719
Graphite Flake/Fog Oil		
35 mg/m^3	3,723,404	2,457
70 mg/m^3	3,625,000	3,177

Figure 12. Graphite flake in feces of a cowbird.
The cowbird was exposed to 35 mg/m^3 of graphite flake 30 minutes per day for 4 consecutive days.

Estimate of Inhalation Dose of Graphite Flake

The amount of graphite flake estimated to deposit in the cowbird lung after 4 exposures was about 0.11 mg/g lung for aerosols containing 35 mg/m^3 graphite flake and 0.25 mg/m^3 for aerosols containing 70 mg/m^3 graphite flake. Single 1-hour exposures to aerosols containing 60 mg/m^3 graphite flake were estimated to deposit about 0.10 mg/g lung in the bird lungs. Red-winged blackbirds exposed to 185 mg/m^3 graphite flake may have received as much as 0.6 mg/g lung. These estimates assume all respirable particles will deposit in the lung and that particle size is not changed during aerosol generation. However, particle coalescence is possible in the presence of fog oil; the deposition may be less in lungs of birds exposed to the cogenerated aerosols.

Response Measures

No mortality, clinical pathology, gross lesions, or behavioral deficits were induced by graphite flake alone or cogenerated with fog oil. Histologic lesions were not clinically significant and appeared to be related to prior antigenic exposure. A small number of birds had fatty liver lesions probably related to diet and onset of ovulation. Minimal to mild anthracosis was observed in treated birds. No tissue damage was evident in the lungs. A WBC response, presumably to the lung-deposited flake, was subclinical at the time of necropsy but was greater in birds exposed to graphite flake without fog oil. Reduced deposition or retention of graphite flake was observed in birds exposed to the cogenerated aerosol. No leukocyte response was observed in birds exposed to the fog oil.

Mortality, Clinical Signs of Toxicity, and Behavioral Abnormalities

Cowbirds exposed for 30 minutes/day for 4 consecutive days to up to 70 mg/m^3 of graphite flake or mixed aerosols of graphite flake and fog oil smoke did not die or exhibit clinical signs of toxicity at anytime during the exposure or post-exposure period. No behavioral abnormalities were observed, including those associated with hypothermia, general stress, and oil-specific postural changes reported for oiled birds or birds fed diets containing petroleum products. Mortality and overt signs of toxicity were also lacking among birds subjected to the single 1-hour exposures. Intense preening was observed in all birds including the exposure controls upon return to the aviary. Aviary control birds did not exhibit this behavior indicating that the behavior was in response to the confinement/handling of the birds during transport and exposure rather than to the test material.

Cowbirds exposed to 35 mg/m^3 graphite flake without fog oil appeared to be caught more readily than birds from the control group ($p \leq 0.04$). These data imply that exposure to graphite flake may affect the vulnerability of cowbirds to predation for several weeks after exposure. However, exposure to a higher concentration of graphite flake (70 mg/m^3) or to the same level (35 mg/m^3) of flake in combination with fog oil did not result in a statistically significant behavioral change. In contrast, cowbirds exposed to 70 mg/m^3 graphite flake cogenerated with fog oil appeared to be captured later in order than control birds ($P \leq 0.01$). Interpreting these data was further complicated by the lack of pre-exposure capture frequency and the capture order for the first two post-exposure captures. In addition, the individual capture order was not complete for each capture event. Also, the initial group assignments may not have been random with respect to capturability. Without this information it is difficult to conclude that the behavioral deficit was induced by exposure to graphite flake rather than individual behavior in repeatedly captured birds.

Capture data for the red-winged blackbirds was more complete and the group populations selected to ensure an equivalent capturability among all groups. Pre-exposure capture order among the blackbirds was found to be similar among all groups. Post-exposure capture probabilities are depicted in Figure 13. Chi-square analysis of the data showed that there was no difference ($P > 0.74$) in capture order between blackbirds exposed to 35 mg/m^3 graphite without fog oil and control birds. No difference ($P > 0.62$) was observed in the vigor of blackbirds exposed to 185 mg/m^3 concentrations of graphite flake cogenerated.

Figure 13. The cumulative probability of capturing a given treated cowbird with respect to five capture order categories.

The first capture category identifies the first 20 percent of a particular treatment group that were captured on any given day. The second category identifies the second 20 percent of a treatment group that were captured on any given day and so forth. Low graphite = 35 mg/m^3; High Graphite-Fog = 185 mg/m^3 flake and 300 mg/m^3 fog oil.

Gross Pathology

All cowbirds were in good body condition at necropsy. No muscular atrophy or depletion of body fat was observed in the control or treated birds. The plumage of the birds exposed to graphite flake and fog oil smoke was in good condition and did not differ in sheen or shape from that of control cowbirds. No evidence of diarrhea or vent staining was found, and no exudates from the eyes or nares were seen. Skin and eye irritation were absent.

The occurrence of gross lesions was few and widely distributed among the treatment groups (Table 7). During necropsy, possible splenic hypertrophy or hyperplasia was noted for 3 cowbirds exposed to 35 mg/m^3 graphite flake (Table 7). Enlarged spleens can indicate a toxin induced immunomodulation in animals (Vos et al. 1998). However, two of the spleens were only marginally enlarged when compared to control spleens on a nonnormalized (to body weight) basis. The third enlarged spleen was congested and came from a bird that had other gross lesions, including an apparent respiratory tract infection. No significant difference in spleen mass was observed between graphite-treated birds and control birds when spleen weight was normalized to percentage body mass (Body Weight, page 49, and Organ Weight, page 51).

Splenic "enlargement" was not seen grossly in birds exposed to the same concentration of graphite flake (35 mg/m^3) when cogenerated with fog oil smoke or in birds exposed to 70 mg/m^3 of graphite flake alone or in combination with fog oil. Further, no treatment-induced histologic abnormalities of the spleen were observed (Histopathology, page 38).

Table 7. Incidence of gross lesions in cowbirds exposed to graphite flake or cogenerated graphite/fog oil smoke.

Treatment (mg/m^3)	Tissues in Which Abnormalities were Found			
	Respiratory	Gonad	Spleen	Peritoneum
4-Day Exposure:				
Graphite Only				
0	--	--	--	--
35	1/8(a)	--	--	--
70	--	--	1/8(b)	--
Graphite/Fog Oil				
35	1/8(c)	1/8(d)	3/8(e)	--
70	--	--	--	--
1-Hour Exposure:				
Graphite Only				
0	--	--	--	--
60	--	--	--	1/5(f)
Graphite/Fog Oil				
60	--	--	--	--

Values are the number of birds with lesion/number of birds in test group.

Exposure concentrations refer to the graphite flake content of aerosol. Fog oil concentrations for the mixed aerosols 121 mg/m^3 for the low graphite 4-day exposures and 103 mg/m^3 for the high 4-day graphite exposures. The fog oil concentration for the single exposure of mixed aerosol was 131 mg/m^3.

(a) Necrotic foci.

(b) Slightly pale.

(c) Petechial and ecchymotic hemorrhages.

(d) Right testis atrophied; lesion occurred in same individual as denoted in (c).

(e) Enlarged. Enlarged spleen from the same individual as denoted in (c) was also congested.

(f) Large hematomain peritoneum (may have occurred during sacrifice).

The spleen of one blackbird appeared to be atrophied at necropsy, but when normalized to the body weight, was not different from the control spleen weight ratios and did not differ from the controls histologically.

The only incidence of gross respiratory lesion seen in the cowbirds was the presence in the air sacs of one bird from the 35 mg/m^3 graphite flake-only exposure group of white buttoned-shaped plaques suggestive of aspergillosis infection. Air sacs and lungs were clear in all other cowbirds.

The majority of gross lesions observed in the red-winged blackbirds were related to viral or fungal infection. The incidence of disease was unrelated to treatment (Table 8). Most blackbirds had mild papular lesions on unfeathered skin around the nares, eyes, and commissures of the beak suggestive of Avianpoxvirus infection. In three cases, the pox-like lesions were more severe and were also found in the buccal cavity. Poxvirus was confirmed histologically (Histopathology, page 38). A probable case of aspergillosis was observed in one individual.

A wide spectrum of gross pathology has been associated with acute toxicity of refined hydrocarbons. The most commonly reported visceral lesion is hemorrhagic gastroenteritis (Crocker et al. 1974, Langenberg and Dein 1982). Other gross lesions associated with petroleum exposure are the same as those reported in birds debilitated by stress and include urate nephropathy and visceral gout, enlarged hemorrhagic adrenal glands, and focal myocardial degeneration (Crocker et al. 1974; Miller et al. 1978a,b; Pattee and Franson 1982; Szaro et al. 1978, 1981). None of these gross lesions were found in the fog oil-exposed cowbirds or blackbirds.

No gross lesions attributable to graphite exposure (e.g., abnormal coloration of the lung) were found in treated birds of either species.

Table 8. Incidence of gross lesions in red-winged blackbirds exposed to graphite flake or cogenerated graphite/fog oil smoke for 4 consecutive days.

Treatment (mg/m^3)	Tissues in Which Abnormalities were Found				Avian Pox Lesion
	Respiratory	Liver	Spleen	Pancreas	
0	--	--	--		5/8
35 (Graphite Only)	1/8(a)		1/8(b)	1/8(c)	7/8
70 (Graphite/Fog Oil)	--	--	--		9/12
Values are the number of birds with lesion/number of birds in test group. (a) Yellow nodules in air sacs (b) Atrophy (c) Slightly pale					

Histopathology

The histological lesions found in the cowbirds and red-winged blackbirds are listed in Tables 9 and 10.

Table 9. Incidence of histopathologic lesions in cowbirds exposed to graphite flake or cogenerated graphite/fog oil smoke.

Treatment (mg/m^3)	Lymphocytic Lesions					Fatty Change of the Liver	Anthracosis
	Liver	Pancreas	Kidney	Adipose	Other		
4-Day Exposure:							
Graphite Only							
0	3/8	1/8	2/8	--	1/8 (a)	1/8	2/8 (f) (g)
35	4/8	2/8	--	--	2/8 (b)	1/8	7/8 (g)
70	4/8	1/8	--	1/6	--	1/8	2/8; 4/8
Graphite/Fog Oil							
35	8/8	1/8	3/8	1/8	2/8 (c)	1/8	5/8
70	3/8	3/8	3/8	--	3/8 (d)	2/8	7/8 (g)
1-Hour Exposure:							
Graphite Only							
0	1/8	--	1/8	--	--	--	0/5 (g)
60	--	--	--	--	2/8 (e)	1/8	5/5 (g)
Graphite/Fog Oil							
60	--	--	--	--	--	--	2/5

Values are the number of birds with lesion/number of birds in test group.

Exposure concentrations refer to the graphite flake content of the aerosol. Fog oil concentrations for the mixed aerosols 121 mg/m^3 for the low graphite 4-day exposures and 103 mg/m^3 for the high 4-day graphite exposures. The fog oil concentration for the single exposure of mixed aerosol was 131 mg/m^3.

(a) Focal granuloma surrounding ova of an helminth parasite.

(b) Granulomatous dermatitis at cloaca; lymphocytic myocarditis and epicarditis.

(c) Multifocal lymphocytic myocarditis; multifocal lymphocytic pericholangitis.

(d) Mild focal lymphocytic pericholangitis; focal lymphocytic myocarditis; mild focal lymphocytic encephalitis.

(e) Lymphocytic pericholangitis; mild lymphocytic adrenalitis.

(f) Ranked on a severity scale of minimum, mild, moderated, and marked, the first ratio is the number of birds with minimum severity anthrocosis (i.e., very few flakes present; no fibrotic tissue changes); second ratio is the number of birds with mild severity anthracosis (i.e. few flakes present, no fibrotic tissue changes). No moderate or marked cases were found. Note that in the two cases of minimum anthracosis in the control group, 1 case of the 35 mg/m^3 graphite-only birds, and 3 cases of the 35 mg/m^3 mixed aerosol exposed birds, the black pigmentation did not appear to be similar in structure to the graphite flake.

(g) Minimal amount of “dust” and/or silica present in the lungs of at least 1 bird of the test group. These contaminants were unrelated to the obscurants.

Table 10. Incidence of histopathologic lesions in red-winged blackbirds exposed to graphite flake or cogenerated graphite/fog oil smoke for 4 consecutive days.

Treatment (mg/m^3)	Hepatitis	Liver/Biliary Hyperplasia	Dermatitis (Avian Pox)	Parasites	Anthracosis
0	--	--	5/8	6/8 (b)	0/8 (d)
35 (Graphite Only)	1/8 (a)	--	7/8	3/8 (b)	4/8; 2/8 (e)
70 (Graphite/Fog Oil)	--	1/8	9/12	5/12 (c)	8/12; 3/12

Values are the number of birds with lesion/number of birds in test group.

(a) Focal granulomatous hepatitis.

(b) Intestinal coccidian.

(c) Coccidia in 5 birds; 1 bird with helminth within the proventriculus.

(d) Ranked on a severity scale of minimum, mild, moderate, and marked, the first ratio is the number of birds with minimum severity anthrocosis (i.e., very few flakes present; no fibrotic tissue changes); second ratio is the number of birds with mild severity anthracosis (i.e. few flakes present, no fibrotic tissue changes). No moderate or marked cases were observed.

(e) Minimal amount of "dust" and/or silica present in the lungs of at least 1 bird in the test group. These contaminants were unrelated to the obscurants.

A large number of the cowbirds had nonspecific, multifocal lymphocytic to granulomatous hepatitis (Figure 14). This lesion was mild to moderate in severity and was likely due to prior antigenic exposure, particularly in response to such infectious agents such as bacteria and mycoplasma species or to parasite migration (Yoder 1984). A few cowbirds had lymphocytic pancreatitis and interstitial nephritis, again nonspecific and probably incidental findings given their broad distribution among treatment groups (including the controls).

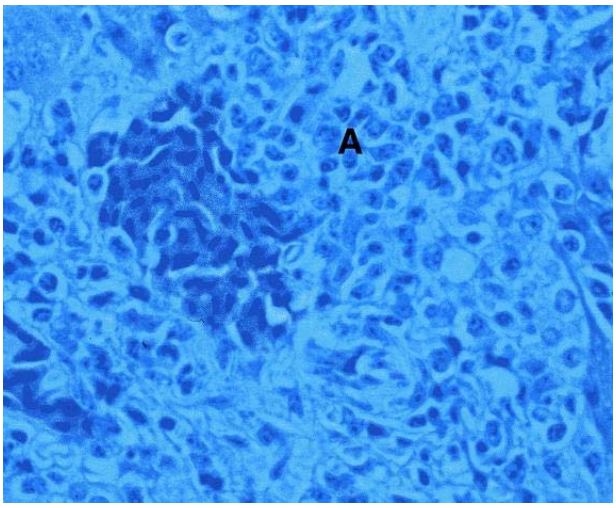


Figure 14. Liver tissue from a control cowbird showing multifocal granulomatous hepatitis.

Mild infections of coccidiosis were observed histologically in most of the cowbirds and blackbirds (Figure 15). Although intestinal coccidiosis was present, no evidence of intestinal damage was observed histologically in infected birds, and it did not con-

found the histopathologic response in this tissue. Coccidial infection was observed in both control and treated birds equally and followed a subclinical course independent of the treatments.

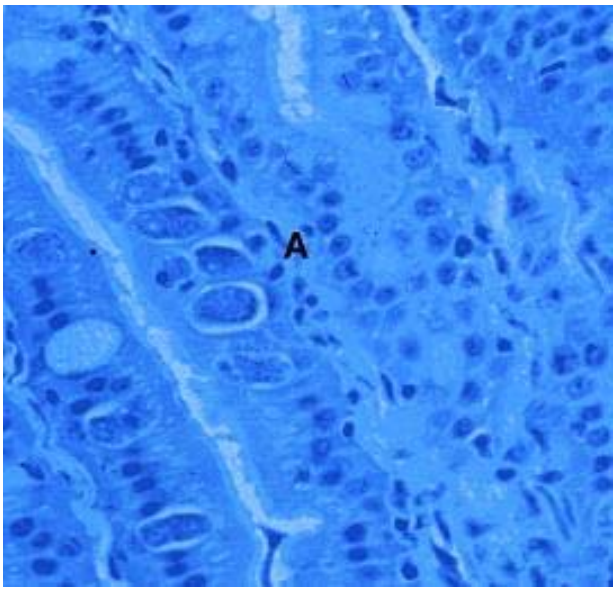


Figure 15. Mild coccidiosis in the intestines of a cowbird.

Only a few blackbirds had nonspecific inflammatory lesions (Table 10). However, proliferative and inflammatory lesions of epithelial tissue due to a poxvirus were observed in birds from all groups. The poxvirus was confirmed by the presence of intracytoplasmic eosinophilic inclusions (Bollinger bodies) in the tissue.

Splenic tissue was normal in all cowbirds. In contrast, the spleens of all the blackbirds (including controls) showed degeneration of lymphocytes within lymphoid follicles. The overall lack of lesions in the blackbirds suggests that this lymphocyte degeneration may be a normal change (apoptosis) representing turnover of lymphoid cells in the spleen.

Fatty change of the liver, a reversible hepatocelluar change, was observed in 7 cowbirds. Mild degenerative changes in the liver, including fatty change, have been observed in a number of wild or experimentally oiled birds (Hartung and Hunt 1966; Beer 1968; Snyder et al. 1973; Szaro et al. 1978, 1981; Pattee and Franson 1982). Although fatty change indicates cell injury, it is not specific for any particular etiology. Metabolic disturbances as well as toxicities can cause this lesion (Lumeij 1994). For example, birds on high energy diets (e.g., mealworms or formulated diet) and limited exercise have developed fatty liver syndrome with the onset of egg production, presumably because of the increase in liver lipogenesis induced by estrogen to supply the developing ova (Jordan 1990). Of the seven cowbirds with fatty change, six were female. Overall, the incidence of fatty liver was low and did not appear to

be related to obscurant exposure (Table 9). No incidence of fatty degeneration of the liver was found in the female red-winged blackbirds exposed to graphite and mixed graphite fog oil aerosols at similar or much higher concentrations (Table 10). Notably, the blackbirds were exposed to the treatments after the end of the reproductive season. Also, fatty livers were not observed in a previous study in which red-winged blackbirds were exposed to 400 mg/m^3 of fog oil for 4 hours (Driver et al. 2002a).

The liver of one red-winged blackbird exposed to a cogenerated aerosol of 185 mg/m^3 graphite flake and 300 mg/m^3 of fog oil had a focus of biliary hyperplasia with a mixed inflammatory cell response including heterophils (Figure 16). The cells of the hyperplastic tissue appeared mature and exhibited little pleiomorphism. No peripheral tissue compression or invasion by the biliary tissue was apparent. Tissue hyperplasia adjacent to inflammatory lesions is common in birds (Campbell 1994). Biliary hyperplasia is also commonly found in birds exposed to aflatoxin, a mycotoxin produced by *Aspergillus flavus* and other fungal species. Aflatoxin is a contaminant found in a wide variety of seeds consumed by birds (Dumonceaux and Harrison 1994). Because tissue hyperplasia adjacent to inflammatory lesions or in response to mycotoxin exposure can be expected in wild caught birds, the isolated incidence of biliary hyperplasia appears to be unrelated to obscurant exposure. With the exception of the fatty degeneration of the liver, none of the wide variety of histologic changes in organ morphology that have been documented in oiled birds (Hartung and Hunt 1966; Beer 1968; Pattee and Franson 1982; Snyder et al. 1973, Szaro et al. 1978, 1981) were observed in the treated cowbirds or blackbirds. The most common lesions reported for oil-treated birds are intestinal necrosis, pneumonia, and renal tubule nephrosis, none of which were observed in the cowbirds or blackbirds exposed to aerosols containing fog oil.

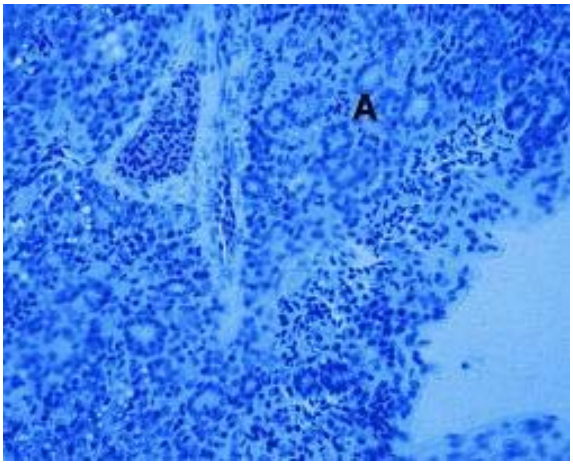


Figure 16. Biliary hyperplasia in liver of a red-winged blackbird.

Anthracosis, the occurrence of exogenous black pigmentation in the lung caused by inhalation of carbon particles, was observed in 67 percent of the cowbirds and 65

percent of the red-winged blackbirds that were exposed to graphite flake alone or in combination with fog oil (Tables 9 and 10). Only 4 percent of the treated cowbirds and 10 percent of the treated blackbirds had sufficient pigment in lung tissue for the lesion to be considered mild. Most of the treated birds had minimal lung pigmentation. Notably, birds exposed to high concentrations of graphite flake had greater incidence of anthracosis and greater severity of the lesion compared to birds exposed to the same concentration of flake in combination with fog oil. This apparent decreased deposition (or greater clearance) of flakes in birds exposed to cogenerated aerosols may be due to particle coalescence/adherence in the presence of fog oil. Coalescence may occur within the plume and/or the nasal turbinates and trachea of the birds. Particle growth is a well documented phenomenon (Green and Lane 1964) and coagulation of multicomponent aerosols has been shown to increase particle size during inhalation in humans (Ingebrethsen 1989). As shown in Figures 11 and 12, the particle size distribution of the graphite flake appeared to change when the flake was cogenerated with fog oil resulting in a somewhat larger AMMD. It is possible that collisions of the flakes with fog oil droplets increased the flake size, or that fog oil coated the graphite flakes and enhanced flake-to-flake coagulation. Particle growth during inhalation was not measured. Such flake growth would result in altered depositional patterns in the respiratory system. In a study using fluorescent microspheres, the nasal operculum in quail (*Colinus virginianus*) chicks was shown to effectively filter out particles greater than about 2 µm in diameter (Driver et al. 1990). Even if the opercula of adult passerines allow particles greater than 2 µm to enter the nasal turbinates, enlarged flakes would deposit in higher regions of the lung or nasal passages than would flakes from the single component aerosol and would be cleared from the respiratory tract by the mucociliary escalator. This rapid clearance of larger particles may be reflected in the greater graphite concentrations in the feces of the birds exposed to the graphite flake cogenerated with fog oil (Estimate of Oral Exposure to Graphite Flake, page 32).

The dark, flake-shaped particles in the cowbird and blackbird lungs were found in the airway epithelial layers and in bronchus associated lymphoid tissue (BALT, Figure 17). The particles were scattered with focally intense accumulations in these areas. This response is expected as the foreign particles are gathered phagocytically and "stored" perivascularly in BALT, or in interstitial tissues, or are cleared from the lungs via the mucociliary escalator (Klika et al. 1997, Scheuermann et al. 1997).

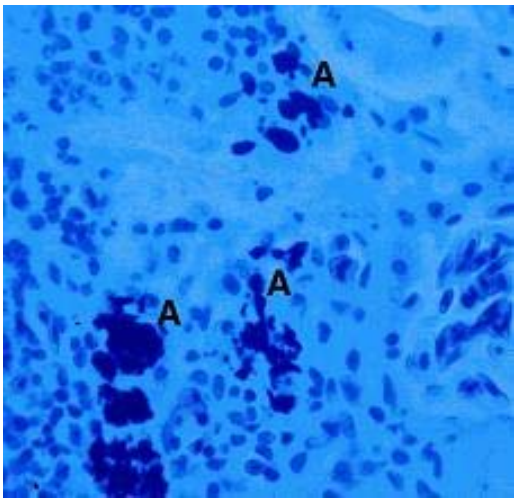


Figure 17. Anthracosis of blackbird lung.
Flakes (A) appear as dark particles. The blackbird was exposed to 185 mg/m^3 of graphite flake co-generated with 300 mg/m^3 of fog oil 30 minutes per day for 4 consecutive days.

Two cowbird controls also had a small amount of dark pigmented material in lung tissue; however, it differed markedly in appearance from the material found in the lung tissue of the treated birds. The pigmented material in the control lungs was globular in form with some refractivity compared to the nonrefractive black flakes seen in the lung tissue of the birds exposed to the obscurant aerosols. The globular material was also found in a few treated birds and appeared to be "dust."

Pigments in the lung can injure or kill macrophages during phagocytosis, releasing compounds that induce fibrosis. If severe enough, the fibrosis can lead to respiratory insufficiency and is suspected of predisposing animals to pulmonary infections (Jones and Hunt 1983). Pulmonary damage has been reported for humans and animals exposed to natural graphite dusts (Gaensler et al. 1966; Klienerman et al. 1979; Martin et al. 1972; Hilscher and Schlipkoter 1972). The reported damage ranges from reticulin deposits to massive fibrosis of the respiratory tissue (Gaensler et al. 1966, Klienerman et al. 1979). These lesions, however, are often attributable to fibrogenic impurities such as silica rather than graphite particles. Synthetic graphite flake of obscurant quality contains only trace quantities of these impurities. The graphite flake in the current study contained about 0.1 percent silica (Table 4), which is below levels that induce silicosis in mammals (Thomson et al. 1986 and 1987, Anderson et al. 1989).

Although normal clearance mechanisms may be capable of removing all of the particles when the exposure is low, repeated or chronic exposure to high concentrations of even low silica carbon particles can overwhelm the lung clearance mechanisms and fibrosis can form in response to the retained particles (Lee 1985, Thomson et al. 1988). There have been no studies which examined lung clearance rates or mechanisms in birds. In mammals, inhalation of large quantities of particles over a long

period of time is required before a moderate lesion develops with corresponding symptoms (Jones and Hunt 1983). The amount of nontoxic dust associated with lung overload appears to be about 1 mg dust/g lung in rats. Retardation of lung clearance, which accelerates the accumulation of dust, occurs at about 0.5 mg dust/g lung in rats (Morrow 1992, Pritchard 1989). The critical deposition rate above which macrophage-mediated lung clearance is overwhelmed has not been determined. If lung clearance in birds is overwhelmed at similar dust concentrations as those for rats, then exposure for 30 minutes to field typical levels (35 mg/m^3) of graphite flake for 8 consecutive days could result in adverse health effects in birds having the same size-adjusted minute volume as cowbirds and female red-winged blackbirds (Table 6). Similarly, significant dust accumulation would be expected at 0.5 mg/g lung with an effective half-life of at least 2 months (Snipes 1989). However, dust retention only 1 month after exposure was minimal in lung tissue of the blackbirds that had estimated dust loadings of 0.6 mg/g lung (Table 6). Although these data may indicate that the threshold concentration is higher for blackbirds, they could also reflect reduced pulmonary deposition of flakes because of particle coagulation in the presence of fog oil aerosol.

A difference in the pulmonary defense system makes it difficult to extrapolate dust-induced effects on lung clearance in mammals to birds. This difference even may provide the avian lung with greater resistance to particulate damage. Recently it was demonstrated that birds lack cells that correspond to the alveolar macrophages of mammals. Instead, the entire epithelial surface in the parabronchi, atria, and part of the infundibulum is capable of taking up particulate matter and transporting the engulfed material to interstitial macrophages (Klicka et al. 1996, Scheuermann et al. 1997). Such an increase is phagocytic area is thought to explain the relatively high resistance of the avian lung to infectious agents (Gerlach 1994) and may be applied to the effective removal of other particles as well.

In the current study, no collagen deposition or alteration of lung structure was observed in either brown-headed cowbirds or red-winged blackbirds that were exposed to aerosols of graphite flake or cogenerated graphite flake and fog oil for 4 consecutive days. At the time of necropsy, there were only minimal amounts of graphite flake in lung tissue and no tissue-level indication of inflammation in the lung. These data indicate that birds can tolerate exposures to graphite flake of up to 185 mg/m^3 for 4 consecutive days without clinically significant damage to the lung when cogenerated with fog oil.

Clinical Pathology

Hematological response to the obscurant exposures was minimal and limited to graphite-only aerosols. No obscurant-induced changes in endpoint metabolites were observed.

Hematology

Hemolytic anemia, a well-documented pathology in birds that have been exposed to petroleum products (Leighton 1982, Leighton et al. 1985) was not observed in any of the treated cowbirds or blackbirds. Erythrocyte morphology was normal in all cowbirds. Only one red-winged blackbird had an abnormal erythron. This bird was exposed to the cogenerated aerosol (185 mg/m^3 graphite flake and 300 mg/m^3 fog oil) and displayed mild (+1) anisocytosis (size variation between red blood cells) and mild polychromasia (color variation between red blood cells). Although increases in erythrocyte polychromasia and anisocytosis are seen in clinical anemia, slight to mild polychromasia, such as observed in this bird, is common and is indicative of normal red blood cell regeneration (Richie et al. 1994). In addition, hematocrit levels for treated red-winged blackbirds were not different from those of controls (Table 11) and were within reference ranges reported for healthy passerines (Altman et al. 1997).

Table 11. Total and differential white blood cell counts for adult female red-winged blackbirds exposed to graphite flake aerosols and cogenerated aerosols of graphite flake and fog oil for 4 consecutive days.

Cell Type[(a)]	Control	Graphite Flake 35 mg/m^3	Graphite Flake/Fog Oil 185 mg/m^3	ANOVA P Value
Heterophile	61.3 (7.9)	57.8 (10.9)	61.1 (10.0)	0.97
Lymphocyte	36.4 (7.5)	38.5 (10.2)	36.9 (9.9)	0.99
Monocyte	2.4 (0.7)	3.5 (1.3)	1.9 (0.4)	0.38
Eosinophile	0	0	0	1.0
Basophile	0	0	0	1.0
Total WBC[(b)]	3000 (327)	3500 (289)	3714 (747)	0.60
PCV	60.3 (5.4)	67.3 (1.7)	64.3 (2.9)	0.58

Values are means with standard error in parentheses.
Fog oil concentration in the cogenerated aerosol was 300 mg/m^3.
(a) Expressed as percentage of total number of WBCs.
(b) Expressed as cells/µL.

No toxic changes were observed in the leukocytes of both cowbirds and blackbirds. WBC counts were within normal ranges reported for passerines (3000 to 9000 cells/µL; Altman et al. 1997). However, the total number of leukocytes in peripheral blood was significantly elevated compared to controls in those cowbirds exposed for 4 days to 70 mg/m^3 of graphite flake or to a single 1-hour exposure of 60 mg/m^3 of graphite flake. Exposure to the same level of graphite flake in combination with fog oil did not result in elevated WBC counts in cowbirds.

Cogeneration with fog oil appeared to reduce the impact of graphite flake on WBC concentrations (Table 12) and paralleled the incidence and severity of anthracosis observed in the birds (see Histopathology, page 38). This reduced response may be a result of decreased deposition of the flake in the lung as a result of particle growth from agglomeration with fog oil droplets and fog oil-coated flakes.

Although WBC numbers were elevated in some test groups, all WBC counts were below levels suggestive of leukocytosis (<10,000 cell/µL) in birds and probably reflect a residual inflammatory response to the foreign material in the lung.

The number of WBCs in the peripheral blood of red-winged blackbirds treated with obscurants was somewhat higher than those in the peripheral blood of control blackbirds, but the differences were not statistically significant (Table 11). The lack of response to a higher graphite flake concentration (185 mg/m^3) than that which elicited a response in the cowbirds (70 mg/m^3) may again be due to particle growth, as a result of particle coagulation (Histopathology, page 38). With a graphite flake concentration of 185 mg/m^3 in the presence of a high fog oil droplet concentration (300 mg/m^3), opportunity for flake-flake agglomeration is greatly increased and probably reflected in decreased deposition of the foreign material in the deep lung.

Table 12. Total and differential white blood cell counts for brown-headed cowbirds exposed to graphite flake aerosols and cogenerated aerosols of graphite flake and fog oil.

Cell Type[a]	Control	Treatment Group (mg/m^3)					
		Four-Day Exposure				Single Exposure	
		Graphite Only		Graphite/Fog Oil		Graphite Only	Graphite/ Fog Oil
		35	70	35	70	60	60
Heterophile	31.4[c] (7.0)	43.8[c,d] (4.2)	52.0[d] (4.2)	32.1[c] (2.7)	48.1[c,d] (4.5)	48.3[c,d] (15.5)	25.7[c,d] (10.8)
Lymphocyte	53.3 (10.0)	55.6 (4.1)	45.7 (3.6)	68.3 (2.9)	50.1 (5.0)	50.7 (14.9)	71.0 (11.1)
Monocyte	1.0 (0.4)	0.6 (0.3)	1.3 (0.6)	0.6 (0.3)	1.7 (0.6)	1.0 (0.6)	3.3 (0.3)
Eosinophile	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)
Basophile	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total WBC[b]	3429[e] (841)	4800[e] (583)	6500[f] (957)	4857[e] (857)	5286[f,e] (834)	7667[f] (1453)	5000[e,f] (2082)

Exposure concentrations refer to the graphite flake content of the aerosol. Fog oil concentrations for the mixed aerosols 121 mg/m^3 for the low graphite 4-day exposures and 103 mg/m^3 for the high 4-day graphite exposures. The fog oil concentration for the single exposure of mixed aerosol was 131 mg/m^3.

(a) Cell types are expressed in percentage of the total number of WBCs.

(b) Expressed as cells/µL.

Different superscripts indicate significant (≤0.05) differences between treatments for each type, Dunnet's T-Test.

Total and differential WBC counts were not depressed in any of the birds exposed to the airborne obscurants (Tables 11 and 12) indicating that the materials did not compromise the nonspecific immune function of the birds.

The number of heterophils in the peripheral blood of the cowbirds was elevated in those birds that were repeatedly exposed to 70 mg/m^3 of graphite flake generated without fog oil. The heterophil response appeared to be the primary contributor to the elevated WBC counts observed in the cowbirds. A single 1-hour exposure to the obscurants did not result in an elevation of this cell type (Table 11).

Adequate thrombocyte counts were found in all birds and no toxic changes were observed in these cells. Also, no blood parasites were found in any of the cowbirds or blackbirds.

Blood Chemistry

Blood chemistries of the cowbirds and blackbirds are shown in Tables 13 and 14, respectively. For both species of birds, exposure to the obscurants did not appear to alter metabolite concentrations in the peripheral blood. All metabolites were within reference ranges that characterize normal organ function in birds (Altman et al. 1997).

No significant differences between the metabolite concentrations of the male and female cowbirds were found, therefore the data were pooled (Table 11).

Table 13. Blood chemistry of adult brown-headed cowbirds exposed to graphite flake aerosols and cogenerated aerosols of graphite flake.

		Treatment Group (mg/m^3)					
		Four-Day Exposure				Single Exposure	
		Graphite Only		Graphite/Fog Oil		Graphite Only	Graphite/Fog Oil
Metabolite[(a)]	Control	35	70	35	70	60	60
Protein	4.9 (0.2)	4.6 (0.4)	4.5 (0.1)	4.9 (0.5)	4.4 (0.2)	4.4 (0.3)	5.5 (0.8)
Albumin	2.7 (0.09)	2.4 (0.11)	2.3 (0.14)	2.4 (0.14)	2.3 (0.08)	2.1 (0.14)	2.6 (0.09)
Globulin	2.1 (0.25)	2.2 (0.35)	2.2 (0.19)	2.2 (0.35)	2.1 (0.13)	2.3 (0.34)	3.0 (0.72)
A/G	1.57 (0.39)	1.25 (0.17)	1.13 (0.17)	1.81 (0.72)	1.01 (0.15)	0.99 (0.16)	0.99 (0.27)
Cholesterol	393 (24)	387 (39)	371 (24)	399 (19)	367 (36)	444 (104)	438 (25)
Uric Acid	11.8 (1.2)	11.5 (1.9)	10.1 (1.7)	11.0 (1.0)	13.5 (1.9)	12.4 (2.1)	11.2 (0.2)
Glucose	424 (23.0)	402 (24.5)	390 (26.2)	376 (24.6)	351 (23.0)	404 (32.5)	378 (37.6)
Calcium	11.1 (1.6)	9.4 (1.7)	16.5 (1.9)	10.2 (1.7)	12.0 (1.6)	14.5 (2.3)	11.5 (2.6)

Values are means with standard errors in parentheses

Exposure concentrations refer to the graphite flake content of the aerosol. Fog oil concentrations for the mixed aerosols 121 mg/m^3 for the low graphite 4-day exposures and 103 mg/m^3 for the high 4-day graphite exposures. The fog oil concentration for the single exposure of mixed aerosol was 131 mg/m^3.

(a) Values for protein are in g/dL; all other metabolites are reported in mg/dL.

Table 14. Blood chemistry of adult female red-winged blackbirds exposed to graphite flake aerosols and cogenerated aerosols of graphite flake and fog oil.

Metabolite[(a)]	Control	Graphite Flake 35 mg/m^3	Graphite Flake/Fog Oil 185 mg/m^3	ANOVA P Value
Protein	5.6 (0.3)	5.0 (0.6)	5.2 (0.6)	0.66
Albumin	2.6 (0.2)	2.2 (0.2)	2.5 (0.3)	0.51
Globulin	3.0 (0.25)	2.8 (0.43)	2.7 (0.29)	0.80
A/G	0.89 (0.05)	0.82 (0.09)	0.94 (0.06)	0.63
Cholesterol	413 (16)	364 (21)	416 (60)	0.69
Uric Acid	8.1 (2.3)	8.2 (2.6)	8.9 (1.2)	0.95
Glucose	227 (51)	359 (42)	215 (53)	0.18
Calcium	11.0 (1.02)	12.7 (0.25)	12.0 (1.37)	0.62

Values are means with standard errors in parentheses.
Fog oil concentration in the cogenerated aerosol was 300 mg/m^3.
(a) Values for protein are in g/dL; all other metabolites are reported in mg/dL.

Although not statistically different, calcium concentrations in female cowbirds (10.3 mg/dL to 16.5 mg/dL) were more variable and somewhat higher than in males (12 mg/dL to 13.4 mg/dL). A slight to moderate increase and variability in calcium levels would be anticipated for female birds approaching the end of breeding season as were the test birds at the conclusion of this study. Also, cholesterol levels for both the cowbirds (367 mg/dL to 444 mg/dL) and blackbirds (364 mg/dL to 416 mg/dL) were elevated over the reference values for passerines (291 mg/dL to 391 mg/dL) but were normal for birds on carnivorous diets. Insects and insect larvae comprised a large portion of the diets of both species in this study.

Body Weight

Mean body weights of brown-headed cowbirds and red-winged blackbirds during the acclimation and test periods are presented in Tables 15 and 16, respectively. No differences in body weight between control and treated birds were observed for either the graphite flake or graphite flake/fog oil exposures throughout the study period in either species. Reports on the effects of oral ingestion of petroleum on body mass and growth in birds are conflicting. The conflicting reports may be a result of the use of different crude oils and refined products, different dosing regimes, and the varying ages of the birds (Clark 1984). Apparently the combined dermal, preening, and inhaled doses in this study were below concentrations that cause weight loss.

Table 15. Mean body weights (in grams) of adult brown-headed cowbirds exposed to graphite flake aerosols and cogenerated aerosols of graphite flake and fog oil.

Day Post-Exposure	Control	Treatment Group (mg/m^3)					
		Four-Day Exposure				Single Exposure	
		Graphite/Fog Oil		Graphite Only		Graphite Only	Graphite/Fog Oil
		35	70	35	70	60	60
28 Days							
Pre-Test	33.0	34.8	36.1	37.0	34.8	41.8	34.0
0	35.1	33.7	42.0	36.7	38.2	39.5	38.1
14	39.1	40.4	43.7	44.3	40.9	47.1	44.0
24	37.8	38.5	42.6	43.7	38.5	40.1	40.7
36	36.2	40.7	42.4	44.2	41.7	47.8	45.3
46	38.2	41.7	42.6	43.7	38.5	40.1	40.7

(a)Exposure concentrations refer to the graphite flake content of the aerosol. Fog oil concentrations for the mixed aerosols 121 mg/m^3 for the low graphite 4-day exposures and 103 mg/m^3 for the high 4-day exposures. The fog oil concentration for the single exposure of mixed aerosol was 131 mg/m^3.

(b) No significant differences in body weight was observed among groups; ANOVA, n=8. The mean body weight of each treatment group was not significantly different (P>0.05) from the control mean; Two-Tailed Dunnett's Test.

Table 16. Mean change in body mass (in grams) of adult female red-winged blackbirds exposed to graphite flake aerosols or cogenerated aerosols of graphite flake and fog oil for 4 consecutive days.

Day Post-Exposure	Control	Graphite Flake 35 mg/m^3	Graphite Flake/Fog Oil 185 mg/m^3	ANOVA P Value
0	58.2	54.5	58.1	0.58
1	53.4	52.1	52.0	0.88
3	48.1	45.4	45.2	0.50
15	50.6	45.4	52.1	0.12
23	50.2	47.0	48.4	0.47
29	47.9	47.5	45.7	0.55
35	45.6	45.1	44.3	0.76

(a) Fog oil concentration in the cogenerated aerosol was 300 mg/m^3.

(b) No significant differences in body weight was observed among groups; ANOVA, n=12. The mean body weight of each treatment group was not significantly different (P>0.05) from the control mean; Two-Tailed Dunnett's Test

Organ Weight

Organ weights (normalized by body weight) of treated cowbirds did not differ significantly from control birds (Tables 17 and 18) and were similar to those reported for healthy birds on a per body weight basis (Miller et al. 1978a,b). Although hypertrophy of hepatic tissue with a single oral dose of petroleum has been demonstrated (Miller et al. 1978a,b; Peakall et al. 1982), the high molecular weight PAHs appear to be the causative constituents of the oils tested (Peakall et al. 1982) and were not detectable in aerosols of fog oil in this study. Changes in spleen mass and structure were not observed providing additional evidence that airborne graphite flake and graphite flake/fog oil mixtures do not induce immune dysfunction.

Table 17. Organ weights (as percentage of body weight) of adult cowbirds exposed to aerosols of graphite flake or cogenerated aerosols of graphite flake and fog oil.

		Treatment Group (mg/m^3)					
		Four-Day Exposure				Single Exposure	
		Graphite Only		Graphite/Fog Oil		Graphite Only	Graphite/Fog Oil
Organ	Control	35	70	35	70	60	60
Liver	3.601[(a)] (0.259)[(b)]	3.439 (0.285)	3.631 (0.647)	3.438 (0.145)	3.219 (0.216)	3.76 (0.467)	3.138 (0.198)
Spleen	0.117 (0.020)	0.231 (0.069)	0.170 (0.020)	0.162 (0.141)	0.189 (0.028)	0.186 (0.111)	0.175 (0.004)
Pancreas	0.333 (0.037)	0.349 (0.034)	0.427 (0.065)	0.320 (0.032)	0.409 (0.029)	0.384 (0.070)	0.359 (0.005)
Kidney	0.435 (0.043)	0.382 (0.051)	0.514 (0.064)	0.506 (0.027)	0.435 (0.034)	0.433 (0.016)	0.457 (0.004)

Exposure concentrations refer to the graphite flake content of the aerosol. Fog oil concentrations for the mixed aerosols 121 mg/m^3 for the low graphite 4-day exposures and 103 mg/m^3 for the high 4-day exposures. The fog oil concentration for the single exposure of mixed aerosol was 131 mg/m^3.

(a) ANOVA was applied to the arcsine transformation values of the tissue weight proportions.

(b) Values in parentheses are the standard error of the mean.

Table 18. Organ weights (as percentage of body weight) of adult red-winged blackbirds exposed to aerosols of graphite flake or cogenerated aerosols of graphite flake and fog oil for 4 consecutive days.

Organ	Control	Treatment Group (mg/m^3)		ANOVA P Value
		Graphite Only	Graphite Flake/Fog Oil	
		35	185 (a)	
Liver	3.142 (0.812)	3.175 (0.432)	2.792 (0.082)	0.296
Spleen	0.101 (0.040)	0.097 (0.016)	0.149 (0.056)	0.68
Pancreas	0.268 (0.048)	0.270 (0.023)	0.237 (0.062)	0.136
Kidney	0.767 (0.144)	0.720 (0.171)	0.808 (0.133)	0.488

(a) Fog oil concentration in the cogenerated aerosol was 300 mg/m^3.
(b) ANOVA was applied to the arcsine transformation values of the tissue weight proportions.
(c) Values in parentheses are the standard error of the mean

Weather Conditions

The birds in this study were maintained outdoors and were subject to field typical weather fluctuations. Cowbirds were exposed to the obscurant aerosols in the spring (Table 19) and experienced temperatures ranging from near freezing to 99°F. Relative humidity ranged from 27 percent to raining during this period. The exposures of the red-winged blackbirds occurred during the fall (Table 19). Temperatures to which they were exposed ranged from below freezing to 83°F.

Table 19. 1998 Weather. April through June represent the exposure and post exposure periods for the cowbirds. October and November represent the exposure and post exposure periods for the red-winged blackbirds.

Month	Temp (F)			RH (%)		
	Min	Max	Mean	Min	Max	Mean
April	34.0	67.4	51.0	26.9	99.6	72.0
May	39.6	92.7	62.6	29.3	99.6	70.0
June	51.8	99.4	72.8	22.8	99.6	55.8
July	60.9	102.4	80.3	21.1	99.6	54.2
August	54.8	106.0	75.1	18.5	99.6	55.7
September	48.5	99.5	70.3	27.0	99.5	87.1
October	27.2	83.4	53.6	31.3	99.6	73.8
November	29.2	68.2	47.5	33.6	99.6	85.4

4 Conclusions

Field typical and higher concentrations of airborne obscurant were generated and characterized. Fog oil composition was changed by the generation process, but the aerosolized oil was not harmful to birds. The effects of airborne graphite flake alone or in combination with fog oil were minimal in brown-headed cowbirds and red-winged blackbirds, the two species of passerine birds used in these studies. Small amounts of graphite flake were observed in lung tissue of treated birds but no tissue damage was observed, indicating that exposure to field typical and higher concentrations of obscurants for 4 consecutive days does not overwhelm the clearance mechanisms of the avian lung. The amount of graphite flake present in lung and feces and the WBC response to the obscurants suggest that the impact of graphite flake is lessened in the presence of fog oil.

No mortality, clinical signs of toxicity, behavioral abnormalities, or poor response to weather fluctuations were induced by obscurant exposure. No cowbirds or blackbirds appeared to have developed gross histological lesions associated with hydrocarbon toxicity or debilitating stress in this study. Evidence of graphite pneumoconiosis was not observed in the birds under gross necropsy nor did the birds appear to exhibit overt respiratory difficulty during or after exposure to the obscurants. Graphite flake was found in the lungs of treated cowbirds and blackbirds but at low levels. No evidence of tissue damage was associated with the graphite retention in the lung. No damage to major organs and tissues from exposure to graphite flake alone or in combination with fog oil was seen. Endpoint metabolite levels in peripheral blood indicated that the functional integrity/capacity of the major organs was intact. Hematological response was normal and no blood cell damage was observed. A mild elevation in WBCs indicative of a past inflammatory response was observed only in birds exposed to graphite flake without fog oil. Total and differential WBC counts indicated that nonspecific immune function was not diminished by the treatments. Spleen weights were also indicative of normal immune function. Further, the incidence of disease and parasitism, which play a role in population regulation, were found to be unrelated to treatment. Thus, it appears that exposure to field typical and high concentrations of graphite flake and fog oil obscurant smoke for 30 minutes/day for 4 consecutive days does not result in mortality, long term organ dysfunction, immunomodulation, behavioral abnormalities, sublethal gross lesions or significant histopathology in brown-headed cowbirds or red-winged blackbirds.

Estimates of flake loading and clearance rate in the lung compared to reported mammalian values suggest avian lung may be more resistant to particulate overload and damage.

No adverse health impacts in birds of similar size would be expected from exposure to graphite flake cogenerated with fog oil at levels less than 200 mg/m^3 graphite flake and a fog oil concentration of at least 100 mg/m^3. Because repeated exposure that may result in particle overload in the lung has the greatest potential for causing harm to birds, it is suggested that exposures at high concentrations be limited to fewer than 8 within a 2-month period. Further information on particle overload phenomena and respiratory clearance of the flakes would be needed to extrapolate other exposure scenarios.

References

Altman, R. B., S. L. Clubb, G. M. Dorrestien, K. Quessenberry. 1997. *Avian Medicine and Surgery.* W. B. Saunders Co., Philadelphia, PA, pp. 1004-1022.

Anderson, R. S., S. M. Thomson, and L. L. Gutshall Jr. 1989. "Comparative effects of inhales silica or synthetic graphite dusts on rat alveolar cells." Arch. Enviro. Contam. Toxicol. Nov;18(6):844-849.

Beer, J. V. 1968. Postmortem findings in oiled auks dying during attempted rehabilitation. In *The Biological Effects of Oil Pollution on Littoral Communities,* J. D. Carthy and D. R. Arthur (eds), pp. 123-129. Field Studies Council, London.

Beletsky, L. D. and Beadle, D. 1996. The Red-winged Blackbird. The Biology of a Strongly Polygamous Songbird. pp. 31-34. Academic Press, Harcourt Brace and Co., Publishers, London.

Bernstein, M. H. 1987. Respiration in flying birds, in T. J. Seller (ed) *Bird Respiration, Vol 11, pp.* 43-74. CRC Press, Inc, Boca Raton, Florida.

Beyer, W. N., G. H. Heinz, and A. W. Redmon-Norwood. 1996. Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. CRC Press, Inc., Florida.

Calder, W. A., and J. R. King. 1974. Thermal and caloric relations of birds. In D. S. Farner and J. R. King (eds), *Avian Biology, Vol IV,* pp.259-413. Academic Press, New York.

Campbell, T. W. 1994. "Hematology'" In *Avian Medicine, Principles and Application. B.* Ritchie, G. Harrison, and L. Harrison (eds.), Wingers Publishing, Inc, Lake Worth, Florida.

Chia, F. S. 1971. Diesel oil spill at Anacortes. *Mar. Pollut. Bull.* 2(7):105-106.

Clark, R. B. 1984. Impact of Oil Pollution on Seabirds. *Environ. Pollution (Series A)* 33:1-22.

Clench, M. H. and R. C. Leberman. 1978. Weights of 151 species of Pennsylvania birds analyzed by month, age, and sex. Bulletin Carnegie Museum of Natural History no. 5, 87 pp.

Connell, C. E., E. P. Odum, and H. Kale. 1960, Fat-free weights of birds. Auk 77:1-9.

Crocker, A. D., J. Cronshaw, and W. N. Holmes. 1974. The effect of crude oil on intestinal absorption in ducklings (Anas platyrhynchos). *Environ. Pollut.* 7:165-177.

Croxall, J. P. 1977. The effects of oil on seabirds. *Rapp. P. -v. Reun. Cons. Int. Explor. Mer.* 171:191-195.

Dein, F. J. 1984. Laboratory Manual of Avian Hematology. Association of Avian Veterinarians, East Northport, New York.

Driver, C. J., L. G. Smith, J. K. Briant, A. E. Jarrell, a. Fairbrother, and P. Bucholz. 1990. "Respiratory exposure of avian wildlife to airborne microbial pest control agents: assessment methodologies." In *Review of Progress in Biotechnology Microbial Pest Control Agent Risk Assessment Program EPA160010-901029* Office of Research and Development, U. S. Environmental Protection Agency.

Driver, C. J., M. W. Ligotke, J. L. Downs, B. L. Tiller, T. M. Poston, E. B. Moore, Jr., and D. A. Cataldo. 1993a. Environmental and Health Effects Review for Obscurant Fog Oil. Report Number ERDEC-CR-071, U.S. Army Chemical and Biological Defense Agency, Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, Maryland.

Driver, C. J., M. W. Ligotke, W. G. Landis, J. L. Downs, B. L. Tiller, E. B. Moore Jr., and D. A. Cataldo. 1993b. Environmental and Health Effects Review for Obscurant Graphite Flakes. ERDEC-CR-056, U.S. Army Chemical and Biological Defense Agency, Edgwood Research, Development and Engineering Center, Aberdeen Proving Ground, Maryland.

Driver, C. J., M. W. Ligotke, H. Galloway-Gorby, G. Dennis, K. A. Reinbold and H. E. Balbach. 2002a. Acute Inhalation Toxicity of Fog Oil Smoke in the Red-winged Blackbird, a Size-specific Inhalation Surrogate for the Red-cockaded Woodpecker. ERDC/CERL Technical Report, TR-02-6, Engineer Research and Development Center, U.S. Army Construction Engineering Research Laboratory, Champaign, Illinois.

Driver, C. J., Ollero, J., Fong Su, Y., Fulton, R., Dennis, G., Tiller, B., Balbach, H., and K. Reinbold. 2002b. Effects of Fog Oil Smoke on the Hatchability and Fledgling Survival of the House Sparrow (*Passer domesticus*), a Nestling Surrogate for the Red-cockaded Woodpecker. ERDC/CERL Technical Report, TR-02-34, Engineer Research and Development Center, U.S. Army Construction Engineering Research Laboratory, Champaign, Illinois.

Driver, C. J., Jarrell, A., Ollero, J., Tiller, G., Fulton, R., Dennis, B., Balbach, H., and K. Reinbold. 2004. Effects of Fog Oil Smoke on Immune Responses in the House Sparrow (*Passer domesticus*) and Red-winged Blackbird (*Agelaius phoeniceus*). ERDC/CERL Technical Report, TR-04-13, Engineer Research and Development Center, U.S. Army Construction Engineering Research Laboratory, Champaign, Illinois.

Dumonceaux, G. and G. J. Harrison. 1994. "Toxins" In In *Avian Medicine, Principles and Application.* B. Ritchie, G. Harrison, and L. Harrison (eds.), Wingers Publishing, Inc, Lake Worth, Florida.

Dunning, J. B., Jr. 1993. CRC Handbook of Avian Body Masses. CRC Press, Boca Raton, Florida.

Fleischer, R. C. and S. I. Rothstein. 1988. Known secondary contact and rapid gene flow among subspecies and dialects in the Brown-headed Cowbird. Evolution 42: 1146–1158.

Fleming, W. J., L. Sileo, and J. C. Franson. 1982. Toxicity of Prudhoe Bay crude oil to sandhill cranes. *J. Wildl. Manage.* 46(2):474-478.

Gaensler, E. A., J. B. Cadigan, A. A. Sasahara, E. O. Fox, and H. E. MacMahon. (1966). Graphite pneumoconiosis of electrotypers. Am. J. Med. 41, 864–882

Gerlach, G. 1994. "Defense mechanisms of the avian host." In *Avian Medicine, Principles and Application.* B. Ritchie, G. Harrison, and L. Harrison (eds.), Wingers Publishing, Inc, Lake Worth, Florida.

Green, H. L., W. R. Lane. 1964. Particulate Clouds, Dusts, Smokes, and Mists: Their Physics and Physical Chemistry and Industrial and Environmental Aspects. Van Nostrand Co., Inc., New York, pp. 138-178.

Harting, R. 1963. Ingestion of oil by waterfowl. Papers of the Michigan Academy of Science 48:49-55.

Hartung, R. 1967. Energy metabolism in oil-covered ducks. *J. Wildl. Manage.* 31:798-804.

Hartung, R., and G. S. Hunt 1966. Toxicity of some oils to waterfowl. *J. Wildl. Manage.* 31:564-570.

Heinz, G. H., and R. W. Johnson. 1982. "Diagnostic brain residues of dieldrin: some new insights." In *Avian and Mammalian Wildlife Toxiciology: Second Conference,* D. W. Lamb and E. E. Kenaga (eds.), ASTM STP 757. American Society for Testing and Materials, Philadelphia.

Hill, E. F. 1994. Acute and subacute toxicology in evaluation of pesticide hazard to avian wildlife. In, *Wildlife Toxicology and Population Modeling: Integrated Studies of Agroecosystems,* R. J. Kendall and T. E. Lacher, Jr. (eds), SETAC Special Publication Series, Lewis Publishers, London.

Hilscher, W. and H. W. Schlipkoter. 1972. Antagonistic factors in the pathogenesis of coal workers' pneumoconiosis. Ann NY Acad Sci 200: 166-181.

Hoffman, D. J. 1979. Embryotoxic and teratogenic effects of petroleum hydrocarbons in mallards. *J. Toxicol. Environ. Health* 5:835-844.

Holcomb, L.C. and G. Twiest. 1968. Red-winged Blackbird nestling growth compared to adult size and differential development of structures. Ohio J. Sci. 68: 277–284.

Ingebrethsen, B. 1989. "The physical properties of mainstream cigarette smoke and their relationship to deposition in the respiratory tract." In *Extrapolation of Dosimetfic Relationships for Inhaled Particles and Gases,* J. D. Crapo, E. D. Smolko, F. H. Miller, J. A. Graham, and A. Wallace Hayes. Academic Press, Inc, San Diego, California.

Jackson, J. A. 1971. The evolution, taxonomy, distribution, past populations and current status of the red-cockaded woodpecker. In *The Ecology and Management of the Red-cockaeded woodpecker: Proceedings of a Symposium at Okefenokee National Wildlife refuge, Folston, Ga, May 26-29,* R. L. Thompson (ed.). Tallahassee, Fl:Tall Timbers Res. Station.

Jackson, J. A. 1994. Red-cockaded Woodpecker (*Picoides borealis*). In The Birds of North America, No. 85 (A. Poole and F. Gill, Eds.). Philadelphia: The Academy of Natural Sciences; Washington, D.C.: The American Ornithologists' Union.

Jones, T.C. and R.D. Hunt. 1983. Veterinary Pathology, Fifth Edition. Lea and Febiger, Philadelphia, PA. p. 74-76.

Jordan, F. T. W. 1990. Nutritional Disorders, In *Poultry Diseases,* 3" Edition, pp. 295-219, Barlbiere Tindall, London.

Kleinerman J., F. Green, R. S. Harley, L. Lapp, W. Laqueur, R. L. Naeye, P. Pratt, G. Taylor, J. Wiot, and J. Wyatt. Pathology standards for coal workers' pneumoconiosis. Arch Pathol Lab Med 103:375-432 (1979).

Klika, E., D. W. Scheuermann, M. H. *A.* De Groodt-Lasseel, 1. Bazantova, and A. Switka. 1997. Lymphatics in the Lung of a Precocial Bird Before and After .Hatching. *Lymphology* 30:173-185.

Klika, E., D. W. Scheuermann, M. H. A. De Groodt-Lasseel, 1. Bazantova, and A. Switka. 1996. Pulmonary macrophages in birds (Barn owl, *Tyto -tyto alba),* domestic fowl *(Gallus gallus f. domestica),* quail *(Coturnix coturnix),* and Pigeons *(Columbia livia). The Anatomical Record.* 246:87-97.

Krahn, M. M., C. A. Wigren, R. W. Pearce, L. K. Moore, and R. G. Bogar. 1988. New HPLC cleanup and revised extraction procedures for organic contaminants. *NOAA Technical Memorandum NMFS F/NWC-153.*

Langenberg, J. A., and F. A. Dein. 1982. Pathology of Ruddy Ducks (Oxyura jamaicensis) Contaminated with Spilled #6 Fuel Oil. In *The Effects of Oil on Birds: Physiological Research, Clinical Applications and Rehabilitation,* D. Rosie and S. N. Barnes (eds), pp. 139-142. Tri-State Bird Rescue and Research, Inc., Wilmington, Delaware.

Lee, K. P., H. J. Trochimowicz, and C. F. Reinhardt. 1985. Pulmonary response of rats exposed to titanium dioxide (TiO2) by inhalation for two years. Toxicol. Appl. Pharmacol. 79, 179–192

Leighton, F. A. 1982. The Pathophysiology of Petroleum Oil Toxicity in Birds: A Review. In *The Effects of Oil on Birds: Physiological Research, Clinical Applications and Rehabilitation,* D. Rosie and S. N. Barnes (eds), pp. 1-28. Tri-State Bird Rescue and Research, Inc., Wilmington, Delaware.

Leighton, F. A., Y. Z. Lee, A. D. Rahimtula, P. J. O'Brien, and D. B. Peakall. 1985. Biochemical and functional disturbances in red blood cells of herring gulls ingesting Prudhoe Bay crude oil. *Toxicol. Appl. Pharmacol.* 81:25-31.

Lipnick, R. L., J. A. Cotruvo, R. N. Hill, R. D, Bruce, K. A. Stitzel, A. P. Walker, I. Chu, M. Goodard, L. Segal., J. A. Springer, and R. C. Myeres. 1995. Comparison of the up and-down, conventional LD50, and fixed dose acute toxicity procedures. Food Chem. Toxicol. 33:223-231.

Lumeij, J. T. 1994. "Nephrology" In *Avian Medicine, Principles and Application.* B. Ritchie, G. Harrison, and L. Harrison (eds.), Wingers Publishing, Inc, Lake Worth, Florida.

Martin J. C., H. Daniel-Moussard, L. Le Bouffant, A. Policard. 1972. The role of quartz in the development of coal workers' pneumoconiosis. Ann NY Acad. Sci 200:127-141.

Miller, D. A., J. Kahn, E. Shaeen, D. B. Peakall, W. B. Kinter. 1978a. Effects of ingestion of a weathered crude oil on immature black guillemots, Cepphus grylle, and herring gulls, Larus argentatus. *Bull. Mt. Desert Is. Biol. Lab.* 17:40-42.

Miller, D. S., D. B. Peakall, and W. B. Kinter. 1978b. Ingestion of crude oil: sublethal effects in herring gull chicks. *Science* 199:315-317.

Morrow, P. E. 1992. Dust overloading of the lungs: update and appraisal. *Toxicol. Appl. Pharm.* 113:1-12.

Moss, 0. R. and Y-S Cheng. 1995. "Generation and characterization of test atmospheres: particles and droplets." In *Concepts in Inhalation Toxicology. Second Edition, R.O.* McClellan and R. F. Henderson (eds.), Taylor and Francis, Washington D.C.

Pattee, 0. H., and J. C. Franson. 1982. Short-term effects of oil ingestion on American kestrels (Falco *sparverius). J. Wildl. Dis.* 18:235-241.

Patton, J. F., and M. P. Dieter. 1980. Effects of petroleum hydrocarbons on hepatic function in the duck. *Comp. Biochem. Physiol.* 65C:33-36.

Peakall, D. B., J. Tremblay, W. B. Kinter, and D. S. Miller. 1981. Endocrine dysfunction in seabirds caused by ingested oil. *Environ. Res.* 24:6-14.

Peakall, D. B., D. J. Hallett, J. R. Bend, G. L. Fourman, and D. S. Miller. 1982. Toxicity of Prudhoe Bay crude oil and its aromatic fractions to nestling herring gulls. *Environ. Res.* 27:206-215.

Phalen, R. F. 1984. Inhalation Studies: Foundations and Techniques. Pp.220-241. CRC Press, Inc., Boca Raton, Florida.

Pritchard, J. N. 1989. Dust overloading-a case for lowering the TLV of nuisance dusts. *J. Aerosol Sci.* 20:1341-1344.

Richie, B. W., G. J. Harrison, and L. R. Harrison. 1994. Avian Medicine: Principles and Application. Wingers Publishing, Inc., Lake Worth, Florida, pp. 865-874.

Schafer E. W. 1972. The acute oral toxicity of 369 pesticidal and pharmaceutical and other chemicals to wild birds. *Toxicol. Applied Pharmacol.* 21:315-330.

Schafer, E. W. 1994. Comparative avian toxicology: What is its role in predicting and monitoring the effect of agricultural pesticides. In, *Wildlife Toxicology and Population Modeling: Integrated Studies of Agroecosystems,* R. J. Kendall and T. E. Lacher, Jr. (Eds), pp.321-338. SETAC Special Publication Series, Lewis Publishers, London.

Schafer, E.W., Jr., W. A. Bowles, Jr., and J. Hurlbut. 1983. The acute oral toxicity, repellency, and hazard potential of 998 chemicals to one or more species of wild and domestic birds. *Arch. Environm. Contam Toxicol.* 12:355-382.

Scheuermann, D. W., E. Klika, M. H. A. De Groodt-Lasseel, 1. Bazantova, and A. Switka. 1997. An electron microscopic study of the parabronchial epithelium in the mature lung of four bird species. *The Anatomical Record.* 249:213-225.

Searcy, W. A. 1979. Sexual selection and body size in male red-winged blackbirds. *Evolution* 33:649-661.

Snipes, M. B. 1989. Long-term retention and clearance of particles inhaled by mammalian species. *Crit. Rev. Toxicol*. 20:175-211.

Snyder, S. B., J. G. Fox, and 0. A. Soare. 1973. Mortalities in Waterfowl Following Bunker C Fuel Exposure. Div. Lab. Animal Med., Stanford Med. Ctr. Stanford, 50 pp.

Stickel, L. F., W. H. Stickel, R. D. McArthur, and D. L. Hughes. 1979. Chordane in birds: A study of lethal residues and loss rates. Toxicology and Occupational Medicine, W.B. Deichmann, ed. Elsevier/North Holland, New York, NY. Pp 387-396.

Stickel, L. F., W. H. Stickel, R. A. Dyranland, and D. L. Hughes. 1983. Oxychlordane, HCS-3260, and nonachlor in birds: lethal residues and loss rates. *J. Toxicol. Environ. Health* 12:611-622.

Stickel, W. H., L. F. Stickel, R. A. Dyrland, and D. L. Huges. 1984. Arocolor 1254 residues in birds: lethal levels and loss rates. *Arch. Envrion. Contam*. Toxicol. 13:7-13.

Szaro, R. C., M. P. Dieter, G. H. Heinz, and J. F. Ferrell. 1978. Effects of chronic ingestion of south Louisiana crude oil on mallard ducklings. *Environ. Res.* 17:426-436.

Szaro, R. C., G. Hensler, and G. H. Heinz. 1981. Effects of chronic ingestion of No.2. Fuel oil on mallard ducklings. *J. Toxicol. Environ. Health.* 7:789-799.

Thomson, S. A., J. D. Bergmann, D. C. Burnett, J. C. Carpin, C. L. Crose, R. J. Hilaski, B. Infiesto, and E. Lawerence-Beckett. 1988. Comparative Inhalation Screen of Titanium Dioxide and Graphite Dusts. Report Number CRDEC-TR-88-88161. U. S. Army Chemical Research, Development and Engineering Center, Aberdeen Proving Ground. Maryland.

Thomson, S. A., D. C. Burnett, R. S. Anderson, and R. J. Hilaski. 1987. Pulmonary and Pathological Responses of Rats to Acute Inhalation of Synthetic Graphite. *Toxicologist* 7:199.

Turcek, E.F. 1966. On plumage quantity in birds. *Ekol. Pol.* Ser. A 14:617-634.

U.S. Army. 1986. *Workshop* to *Select Test Systems for Ecological Assessment and Monitoring.* January 19-22, 1986, U.S. Army Construction Engineering Research Laboratory (CERL), University *of* Illinois at Urbana-Champaign, Illinois.

Van Velzen, A. C., W. B. Stiles, and L. F. Stickel. 1972. Lethal mobilization of DDT by cowbirds. *J. Wildl. Manage.* 36:733-739.

Vos, J. G., 1. Kdimber, C. Kuper, H. Van Loveren, and H. Schuurman. 1998. The Immune System, In: *Target Organ Pathology,* J. Turton and J. Hooson eds, pp 207-237, Taylor and Francis, Bristol, Pennsylvania.

Walsberg, G. E., and J. R. King. 1978. The relationship of external surface area of birds to skin surface area and body mass. *J. Exp. Biol.* 76:185-189.

Weatherhead, P. J. and K. L. Teather. 1987. The paradox of age-related dominance in Brown-headed Cow-birds (*Molothrus ater*). Can. J. Zool. 65: 2354–2389.

Yoder, H. W. 1984. Avian Mycoplasmosis In M. S. Hofstad, H. J. Barnes, B. W. Calnek, W. M. Reider, and H. W. Yoder, Jr. (ed), *Diseases of Poultry,* Eighth Edition, pp.193-195. Iowa State University Press, Ames, Iowa.

Zar, H. H. 1974. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE (DD-MM-YYYY)
01-2005

2. REPORT TYPE
Final

3. DATES COVERED (From - To)

4. TITLE AND SUBTITLE
Inhalation Toxicity of Cogenerated Graphite Flake and Fog Oil Smoke in the Brown-headed Cowbird and the Red-winged Blackbird, Size-specific Inhalation Surrogates for the Red-cockaded Woodpecker

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Crystal Driver, Robert Fulton, Jennifer Ollero, Mark Clark, Gary Dennis, Brett Tiller, and Harold E. Balbach

5d. PROJECT NUMBER
SERDP

5e. TASK NUMBER
CS-507; F474HJ

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
U.S. Army Engineer Research and Development Center (ERDC)
Construction Engineering Research Laboratory (CERL)
PO Box 9005
Champaign, IL 61826-9005

8. PERFORMING ORGANIZATION REPORT NUMBER
ERDC/CERL TR-05-5

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Strategic Environmental Research and Development Program
901 N Stuart St., Suite 303
Arlington, VA 22203

U.S. Army Forces Command
Fort McPherson, GA 30330

10. SPONSOR/MONITOR'S ACRONYM(S)
Program Manager

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
Copies are available from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

14. ABSTRACT

The red-cockaded woodpecker (*Picoides borealis*) is an endangered species found on many installations where troop readiness training is conducted. Conducting maneuvers under obscurant cover is important for realistic training. Generators that combine fog oil for visual obscuration with graphite flakes for infrared obscuration are being deployed for training scenarios. The effect of this combination on avian species was unknown. Our data indicate that toxicity of inhaled and/or preened graphite flake and cogenerated graphite flake and fog oil is low and similar to controls for adult cowbird and blackbird surrogates for the red-cockaded woodpecker. No mortality, body weight loss, clinical signs of toxicity, or respiratory function deficits were observed in the graphite flake-only, or cogenerated graphite flake/fog oil-treated birds when compared to controls. Hematological response was normal and no toxic effects in erythrocytes or leukocytes were found. White blood cell counts, spleen weights, and incidence of parasitism and disease were indicative of normal immune function in all treatments. Because repeated exposure that may result in particle overload in the lung has the greatest potential for causing harm to birds, it is suggested that exposures to high concentrations of the aerosol-graphite mixture be limited to fewer than eight within any 2-month period.

15. SUBJECT TERMS
fog oil
red-cockaded woodpecker
endangered species
smoke and obscurants
natural resource management
military training

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Harold E. Balbach
Unclassified	Unclassified	Unclassified	SAR	74	19b. TELEPHONE NUMBER (include area code) 217-352-6511, ext 6785